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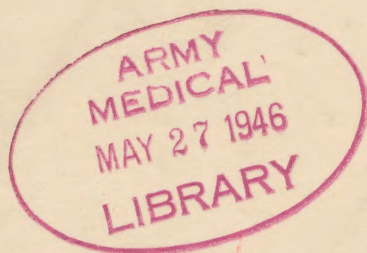
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ARMORED MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

INDEXED



Final Report

On

PROJECT NO. 18 - INVESTIGATION OF THE EFFECTS OF ACTIVITY AND
ENVIRONMENT ON ATABRINE THERAPY

Project No. 18

INFORMATION COPY

23 December 1943

ARMORED MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky

Project No. 18
441.1 GNOML

23 December 1943

1. PROJECT: No. 18 - Final Report on, Investigation of the Effects of Activity and Environment on Atabrine Therapy.

a. Authority - Reference 3rd Indorsement, Headquarters Army Ground Forces, Army War College, Washington, D. C., file 720 (12 July 1943) GNMED, Dated 31 July 1943. Subject: Investigation of Atabrine Blood Levels at Armored Medical Research Laboratory, Fort Knox, Kentucky.

b. Purposes -

- (1) To determine with each of two recommended suppressive regimens administered to troops during active training:
(a) The time required to develop equilibrium plasma atabrine levels; (b) the magnitude of the level in relation to dosage and (c) the variability in levels among individuals on the same regimen.
- (2) To determine the effect of high initial doses in shortening the time required to reach equilibrium levels.
- (3) To determine the effects of a simulated jungle climate upon the plasma atabrine levels.
- (4) To study the rate of reduction of plasma atabrine levels after discontinuing administration of the drug.
- (5) To study the relationship between the plasma atabrine levels obtained with therapeutic doses and those established previously with suppressive regimens.

2. DISCUSSION:

a. Two hundred and fifty men were employed who were subdivided for purposes of study, as follows: Two groups of 100 men each (from the Armored Replacement Training Center) were given 0.4 and 0.6 gm atabrine per week respectively, a third group of 30 on 0.6 gm/wk was rotated through the Laboratory hot room and a fourth group of 20 men was used for special studies.

b. Details of allocation, conditions, dosage regimens and sampling schedules and results are given in the appendices.

c. The analytical method employed was that described by Brodie and Shannon - Malaria Report No. 9, NRC.

d. Experience with the drug, the men, and the chemical method has led to certain conclusions. In addition, one arrives at inferences with respect to the application of this experience to the continued investigation of this problem. These points of view are summarized and from them the recommendations have evolved.

e. A table of contents follows this section.

3. CONCLUSIONS:

a. Plasma atabrine levels obtained with two constant regimens.

- (1) The group plasma level at any time on a given regimen is a function of the daily dose, the pre-existing level and the time interval since the last dose.
- (2) The group mean level attained on a given regimen rises progressively for from four to eight weeks and finally reaches an equilibrium level which remains substantially constant thereafter.
- (3) The group mean equilibrium level is directly proportional to the average daily dose. With dosages of 0.6 gm/wk, a mean plasma level of 17 micrograms/L is reached-- and with 0.4 gm/wk, a level of 12 micrograms/L.
- (4) Individual plasma concentrations obtained with a given regimen differ widely. In a group exhibiting a mean equilibrium level of 12 micrograms/L, individual concentrations varied from 5 to 85 micrograms/L.

b. Plasma atabrine levels with regimens instituted after high initial doses.

- (1) The time required to reach the equilibrium level characteristic of any regimen can be greatly reduced by administration of larger doses prior to initiation of the maintenance regimen.
- (2) The final equilibrium level attained with the subsequent maintenance dose is not altered by initial priming doses of short duration.

c. Effects of simulated jungle climate.

- (1) The group equilibrium level achieved with a given dosage regimen in a hot humid environment is the same as that obtained at normal temperatures.

- (2) Rate of acclimatization and performance of men in a hot humid environment are not affected adversely by suppressive therapy.

d. Rate of decrease of group mean plasma level when administration of the drug is discontinued. When the drug is no longer taken the concentration of plasma atabrine falls, the level at any time thereafter being a function of the pre-existing level and the time interval since discontinuing dosage. The level existing at any time is reduced by approximately 10% in the ensuing 24 hours.

e. Level obtained with therapeutic dosage.

- (1) Administration of therapeutic doses for one week to men at equilibrium with suppressive regimens results in a degree of rise of plasma level which is a function of the added quantity administered and the pre-existing level.
- (2) Men who are characterized by low plasma levels while on suppressive dosage regimens will in general have relatively low levels when on standard therapeutic regimens.

f. Analytical method.

- (1) The precision of the method as employed is within ± 2 micrograms/L.
- (2) Larger errors which affect all determinations in a single day may occur.
- (3) The relative precision of the method is greatest at the higher levels. For plasma levels below 10 micrograms/L the method must be used with extreme care.

g. Toxicity of atabrine. No unequivocal toxic reactions occurred in any of the 250 men studied for three months.

4. RECOMMENDATIONS:

a. That the following studies be expedited:

- (1) Field investigations in hyperendemic areas to determine the minimum plasma atabrine level required for the suppression of malaria.
- (2) Further investigation, in the individual, of the value of the plasma atabrine level and other indices, as measures of antimalarial protection.
- (3) Laboratory studies to improve the chemical method and equipment.
- (4) A controlled field study of the toxicity of atabrine.

b. The minimum suppressive plasma atabrine level has not yet been established. Breakthroughs have occurred with weekly dosage of 0.4 gm. In the present study no toxic reactions occurred with this or larger suppressive doses. Pending results of the suggested studies, therefore, it is recommended that the dosage regimen for troops entering hyperendemic theaters be established as follows:

- (1) 0.1 gm atabrine twice daily after meals for 1 week, to be given not later than the week immediately preceding exposure.
- (2) 0.1 gm. atabrine daily thereafter so long as exposed.

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APPENDIX A

SUMMARY OF RESULTS

1. Administration of atabrine under a given dosage regimen results in a progressive increase in plasma concentration until an equilibrium level is reached which continues thereafter, with little variation, so long as the dosage schedule is maintained. After discontinuing the drug, the plasma level decreases rapidly at first and then more and more slowly. There are marked variations in plasma levels among the individuals of a group subjected to the same regimen, but with few exceptions they follow a pattern similar to that exhibited by the group as a whole. This characteristic pattern - the progressive buildup, the equilibrium and the dieaway - appears to be fixed by a simple law which makes it possible to predict the course which the mean concentration of a group will follow when subjected to a given dosage regimen. The extent of the individual departure from this mean value is also predictable. These and other important behavior characteristics exhibited by a group of men are considered below. The procedure employed and more detailed considerations of the results are presented in subsequent sections.

2. Buildup of plasma atabrine concentration.

Although different dosage regimens, in which the administration is intermittent, exhibit characteristic detailed differences in pattern, the regular repetition of dosages each succeeding week, results in a general rising course which is susceptible to simple description. The plasma concentration increases rapidly during the first week and then more and more slowly as equilibrium is approached. At the end of the first week approximately one-half of the final equilibrium level is attained; at the end of the second week, three-fourths of the equilibrium level; at the end of the third week, 87%; at the end of the fourth week, 94% and so on; each week halving the remaining difference so that by the end of the sixth week no perceptible difference from equilibrium is observed.

3. Equilibrium level attained in relation to dosage regimen.

At equilibrium the average concentration resulting from administration of a specific amount of drug each week depends upon the total weekly dose. Representative values for a group are found to be directly proportional to the dose. Thus for the two regimens, 0.4 gm/wk and 0.6 gm/wk, the group mean equilibrium levels attained were 12 and 17 micrograms/L, respectively. In view of this direct proportionality, it is a simple matter to calculate, within the general limits of the study, the predicted equilibrium plasma level for other dosage regimens. The relationship is: 30 micrograms/L for 1 gm weekly dose. (Total)

4. Effect of large initial doses upon the time required to reach maintenance levels.

As was pointed out in par. 2 above, from 4 to 6 weeks are required on a regular weekly regimen to attain the resulting equilibrium. However, a practical expedient immediately suggests itself whereby a desired maintenance level can be obtained within a few days. Since half-equilibrium values are reached with any regimen in 1 week, if double the maintenance dosage is given in the first week the level reached at the end of this time will be same as the equilibrium level of the maintenance dose. This avoids from 3-5 weeks delay. By administration of 3 times the maintenance dose initially, the required level will be reached after four daily doses; with 4 times the maintenance dose, in 3 days, etc.

5. Decrease of plasma atabrine concentration after discontinuing the drug.

When the drug is discontinued after the establishment of an equilibrium state the plasma atabrine concentration decreases in a manner very similar to the initial buildup. Thus, 10% of the level is lost each day and at the end of a week the level will have dropped to half the equilibrium value, to one-fourth in two weeks, one-eighth in three weeks and so on, each week halving the remaining concentration.

6. Effect of climatic environment upon equilibrium level.

An important phase of the present study was to determine the effect, if any, of a hot, humid environment upon the course of buildup, and upon the equilibrium level attained with a given regimen as compared with the equilibrium level reached with the same dosage schedule in a temperate climate. The results, as indicated in the table below, show that climate had no influence upon the equilibrium plasma atabrine level.

TABLE 1

EQUILIBRIUM PLASMA ATABRINE LEVELS ATTAINED UNDER DIFFERENT
CLIMATIC EXPOSURES WITH SAME DOSAGE SCHEDULE -
0.6 GM PER WEEK

Exposure	No. of Results	No. Men	Group Mean \bar{C} Concentration 7th Through 11th Weeks	Standard Geometric Deviation
Outside, A.R.T.C.	358	85	$17.2 \times \frac{x}{7} 1.04$	1.44
7 wks jungle, 4 wks outside Group A	205	15	$19.9 \times \frac{x}{7} 1.17$	1.41
7 wks outside, 4 wks jungle Group B	205	15	$19.9 \times \frac{x}{7} 1.08$	1.32

It was also found, in this phase of the study, that neither the administration of atabrine concurrent with initial exposure to a hot, humid environment, nor the establishment of a suppressive atabrine level prior to such exposure, in any way affected the work capacity of the subjects in the heat nor their rate of acclimatization.

7. Plasma levels attained with the therapeutic dosage schedule.

The increase of plasma concentration with the institution of a therapeutic regimen after the establishment of equilibrium under a suppressive dosage schedule can be described by the same expressions that were derived from the behavior of the drug at the lower dosage rates. The concentration approaches a new equilibrium level which is directly proportional to the dosage and the manner of increase is the same as before. It was found possible to predict the plasma level attained with therapeutic dosages in the same manner and with equal success as was the case with the concentrations under the suppressive regimens, when due account was taken of the transients.

8. Individual variability.

The difference between plasma levels in different individuals may be great. This variability follows a regular pattern and is consistent with the type of statistical analysis (geometric) to which the data have been subjected. Description of the statistical procedure will be given in the next section. A possible biochemical explanation of the wide differences between individual results is offered. It is believed that variation in protein binding is the main cause of the wide ranges in values. The atabrine concentration in erythrocytes varied independently of plasma atabrine level and was more nearly proportional to dosage experience. It is inferred from this that a variation in partition coefficient rather than differences in total quantities involved may be responsible for the variance. Evidence on this was not conclusive.

APPENDIX B

METHODS AND RESULTS OF MATHEMATICAL TREATMENT OF DATA

SECTION I - Statistical Analysis of Individual Variability.

1. Distribution of individual plasma levels.

Individuals on the same dosage regimen differ markedly in their plasma concentrations. For example, a group of 85 men on a 0.4 gm/wk regimen were distributed with respect to their plasma levels as shown in Chart 1. In order to describe the pattern of variability statistical treatment of the data has been employed. It is immediately evident from the unsymmetrical pattern shown in Chart 1 that the variability does not follow the normal arithmetic probability distribution. The arithmetic mean and standard deviation, which apply to the normal probability curve, cannot be employed, therefore, to describe the distribution of individual plasma atabrine levels. When the variations in atabrine concentrations are expressed logarithmically, however, the distribution is approximately symmetrical, and is found to approach closely a logarithmic probability distribution, as shown in chart 2. The normality of the logarithmic distribution is demonstrated by the fact that the cumulative frequency curve plotted on logarithmic probability paper yields an approximately straight line over most of the range, as seen in Chart 3. This linearity test was applied to every set of group data and satisfactory straight-line plots were obtained in all cases. It should be pointed out, that a few exceptional individuals exhibited abnormally high atabrine levels for extended periods. The number is not sufficient, however, to affect appreciably the group values.

2. Significance of geometric distribution.

The representative value for a group which is characterized by a logarithmic probability distribution is the geometric mean (anti-log of the arithmetic mean of the logarithms of the plasma atabrine values in a group of data) instead of the customary arithmetic mean, and the dispersion of the data is measured by the standard geometric deviation rather than by the standard deviation of the arithmetic values. The concept of dispersion as measured by the standard geometric deviation (anti-log of the standard arithmetic deviation of the logarithmic plasma levels about the logarithmic mean) differs basically from the common conception of linear dispersion, measured by the arithmetic standard deviation. In the case of a normal arithmetic distribution, probabilities are equal, that deviations of ± 1.0 standard deviation about the mean will occur. With a log-probability distribution, on the other hand, probabilities are equal, that deviations obtained by multiplying or dividing by (1.0) one standard geometric deviation, will occur. Thus, in

CHART - I

FREQUENCY DISTRIBUTION OF PLASMA ATABRINE LEVELS IN CO. B SECTION I. 7 TH THRU 11TH WEEK

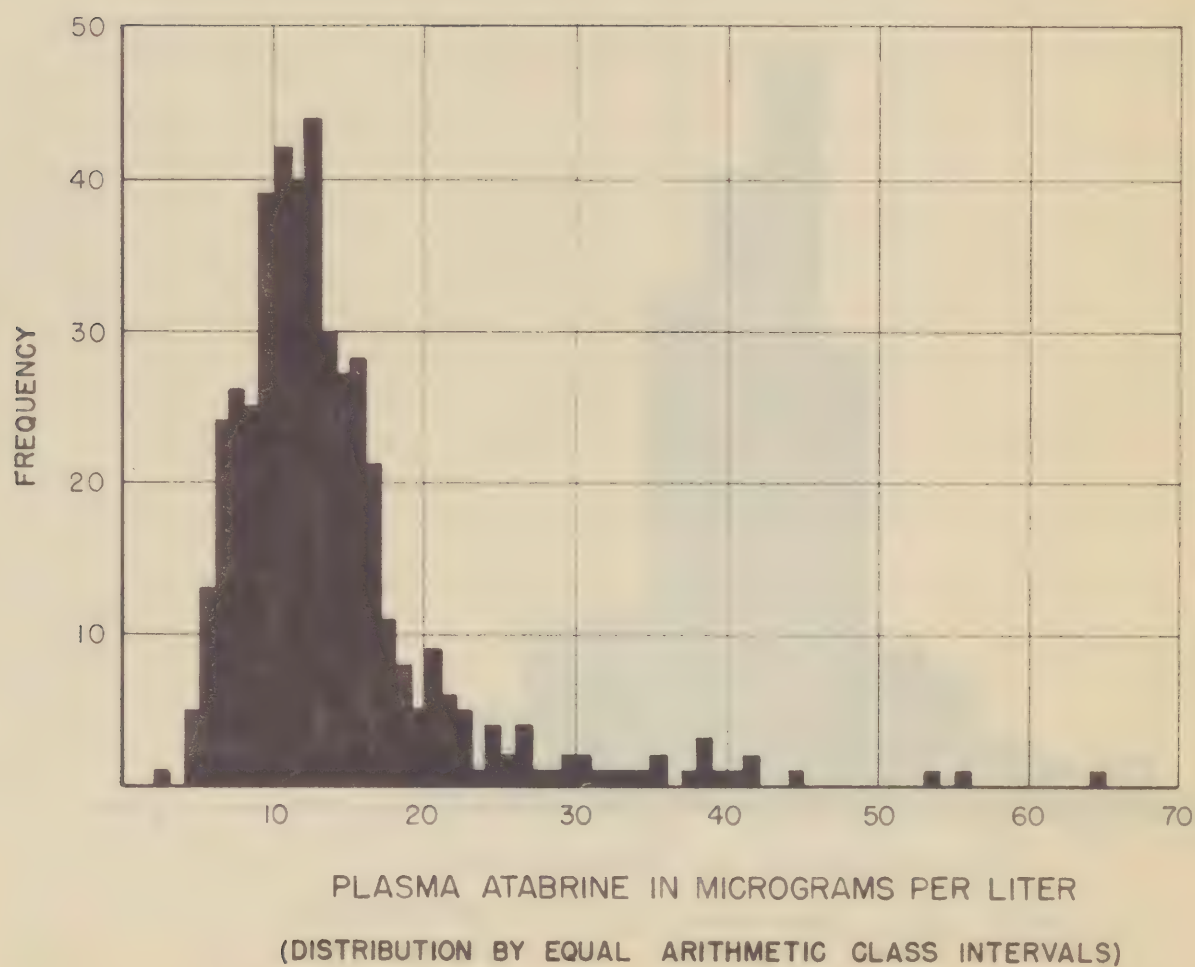


CHART-2

FREQUENCY DISTRIBUTION OF PLASMA ATABRINE LEVELS
IN CO. B, SECTION 1, 7TH THRU 11TH WEEK

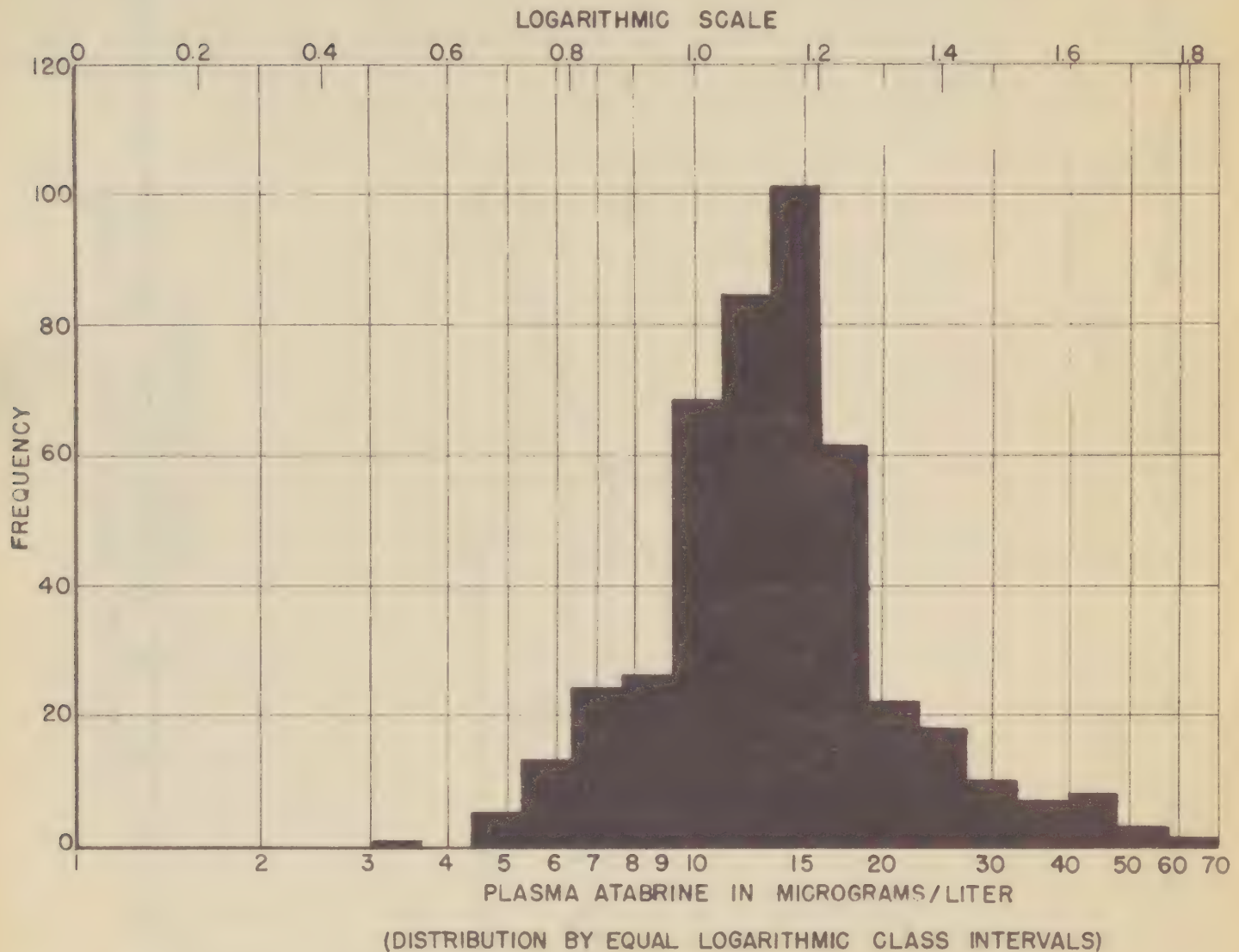


CHART-2

CUMULATIVE PERCENTAGE DISTRIBUTION OF PLASMA ATABRINE LEVELS

CO. B, SECTION I, 7TH THRU 11TH WEEK

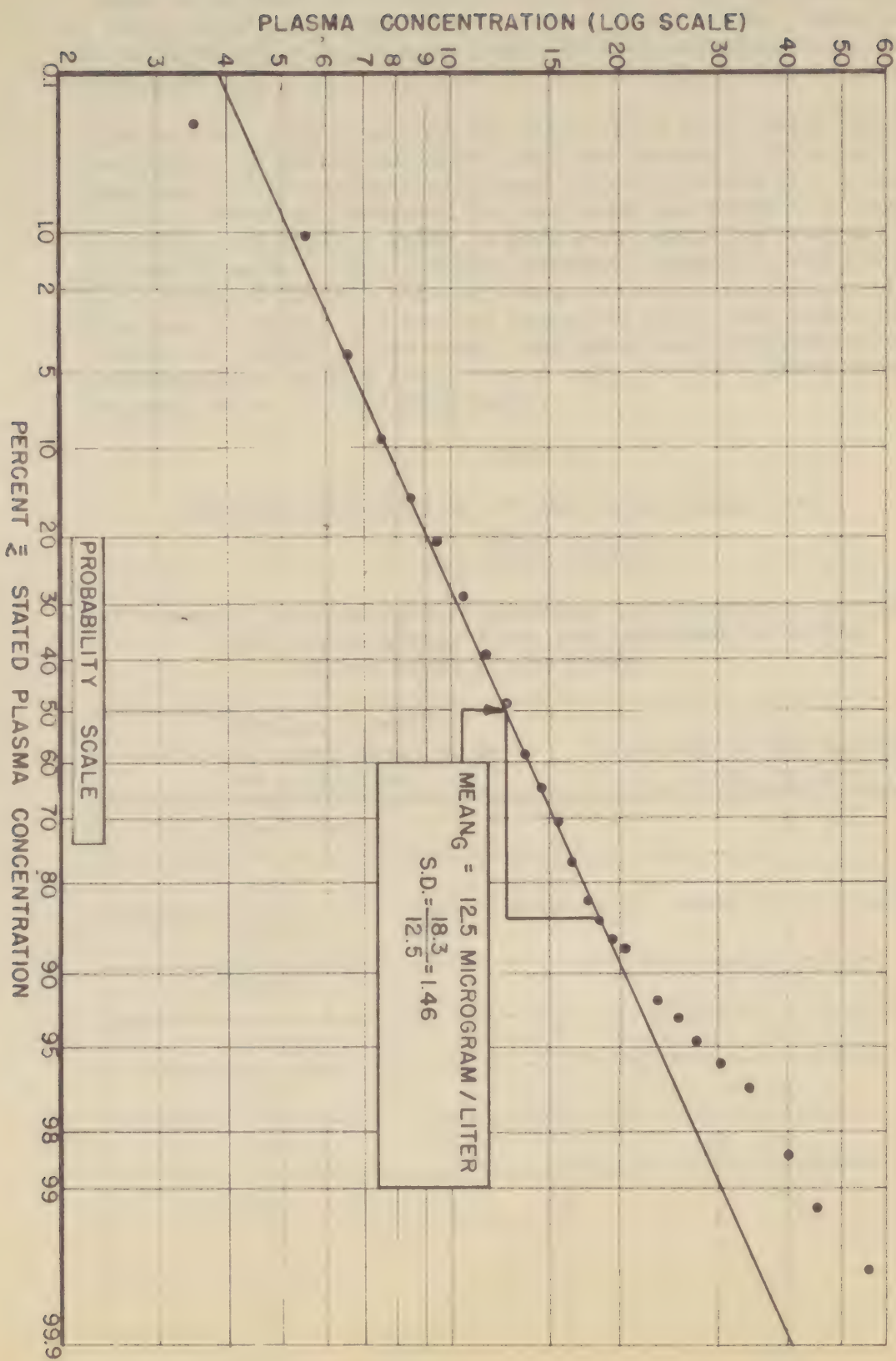


CHART-3

terms of a normal logarithmic probability curve, the relative significance of high and low values becomes quite different from their meaning when viewed in arithmetic relation to the mean. Under the geometric concept, a level of 80 micrograms/L in a group of data having a mean level of 20 micrograms/L has the same probability of occurrence as a level of 5 micrograms ($\frac{80}{20} = \frac{20}{5} = 4$), whereas, in a normal arithmetic probability distribution, with a mean of 20, values of 5 would occur with much greater frequency. It is not suggested that the geometric distribution is an invariable characteristic of plasma atabrine levels of a group. It was, however, repeatedly observed in this study and provides a method for describing variability which is more representative than is the common arithmetic method. The standard geometric deviation was found to be quite constant for all sets of data, exhibiting relatively little variation from week to week. Its average value was 1.45, from which one may predict (within the limits of the study) the range and distribution of plasma concentrations among a group of men subjected to a given suppressive regimen, as in the following table:

TABLE 2
PREDICTED DISTRIBUTION OF INDIVIDUAL PLASMA LEVELS
IN
MEN ON A GIVEN REGIMEN

(Presented in a cumulative frequency distribution of the individual plasma levels which are expressed as ratios of the Mean_G of the group)

Individual Levels (Factor by which Mean _G is to be multiplied)	Percentage of Men Having Stated Level or Less
0.4 x Mean _G	1
0.5	3
0.6	8
0.7	17
0.8	27
1.0	50
1.2	70
1.4	82
1.6	90
2.0	97
2.5	99

APPENDIX B

SECTION II - Prediction of Group Plasma Atabrine Levels.

1. Discussion.

a. It is the purpose of this section to describe the simple expressions by means of which representative group values can be predicted and to present the derivation of these expressions for the observed data.

b. The plasma value which is representative of a group has been shown in the previous section to be the geometric mean (Mean_G) of the individual plasma atabrine concentration. This section will be devoted entirely to the consideration of such representative group values.

2. Concept of transients and underlying levels.

Following the administration of atabrine the concentration of the drug in the plasma exhibits rapid changes associated with the entrance and redistribution of the drug. After twenty four hours, however, these initial disturbances, have practically disappeared and the then observed values can be very simply correlated. For the purpose of this discussion, the initial disturbances will be spoken of as transients and the term underlying level will be applied to all values found more than 24 hours after administration of any dose. The underlying level may be thought of as existing at all times but concealed by the presence of the transients during the first day following administration. The first part of the analysis will deal with the correlation of data on underlying levels and the second part with the evaluation of the transients. Knowledge of the transients becomes particularly necessary during a therapeutic regimen where the doses are administered at intervals of less than a day.

3. Simple mechanism correlating underlying levels.

a. The most important fact which emerges from a study of the data on underlying levels is that all the values observed can be computed, within experimental error, from two constants. This implies a simple mechanism which may be expressed as follows:

b. The net change in underlying plasma level is the result of -

(1) A gain in concentration directly proportional to the dose - 23.2 micrograms/L for 1 gm dose.

(2) A loss in concentration proportional to the existing

concentration and time interval - 10% of concentration per day.

4. Computation of underlying level for a daily dose regimen.

a. In order to determine the pre-dose level on any day from the pre-dose level of the previous day, the procedure is as follows:

- (1) To the pre-dose value of the first day add the contribution of the new dose - 23.2 times dose in gm.
- (2) From this new value subtract 10% of the new value, thus giving the pre-dose level for the second day in micrograms/L.

b. By means of this procedure, curves have been built up day by day for each of the groups.

c. In Charts 4 and 5, the observed values for the groups are shown by the dotted lines, for comparison with the computed values, solid lines. In Chart 6 the values are shown for a group (Jungle) which was given 1.2 gm in the first week and 0.6 gm thereafter. Calculated values are indicated by the solid curve and the observed by the dotted curve.

d. Tables 3, 4, 5 and 6* give the computations for all curves. Calculations for the therapeutic levels will be considered later. Summary of the more detailed step-by-step calculations for the groups is given in Chart 7.

e. This procedure may be expressed algebraically -

$$L' = L + AD - K (L + AD)$$

where L = group mean_G plasma concentration just before a dose.

L' = same for succeeding day.

D = dose in gm.

A = gain per gm of dose = 23.2 micrograms/L/gm.

K = percent of level lost per day = 10%.

Then

$$L' = (1-K) (L + AD) = 0.9 (L + 23.2D)$$

(D may be zero on days of no dose)

The procedure of adding the gain immediately at the time of administration has been adopted arbitrarily for simplicity and to partially offset

* Tables will be found at the end of this section.

COMPARISON OF THEORETICAL AND EXPERIMENTAL PLASMA ATABRINE LEVELS

0.4 GM. PER WEEK

EXPERIMENTAL CURVE BASED ON THE RESULTS FOR 85 MEN

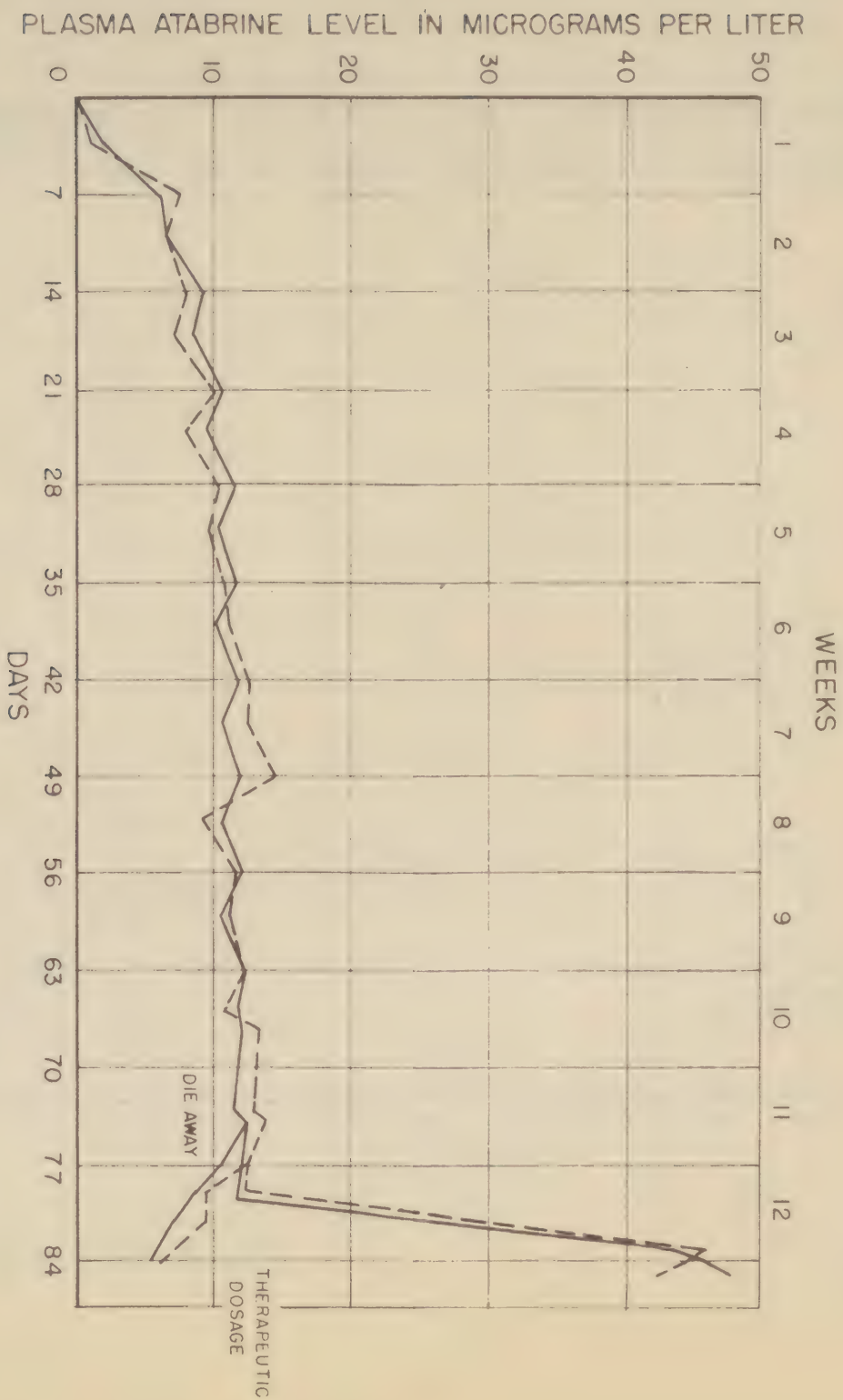


CHART - 4

CHART —5

COMPARISON OF THEORETICAL AND EXPERIMENTAL MEAN₆ PLASMA ATABRINE LEVELS ON A DOSAGE SCHEDULE OF 0.60 GRAMS PER WEEK

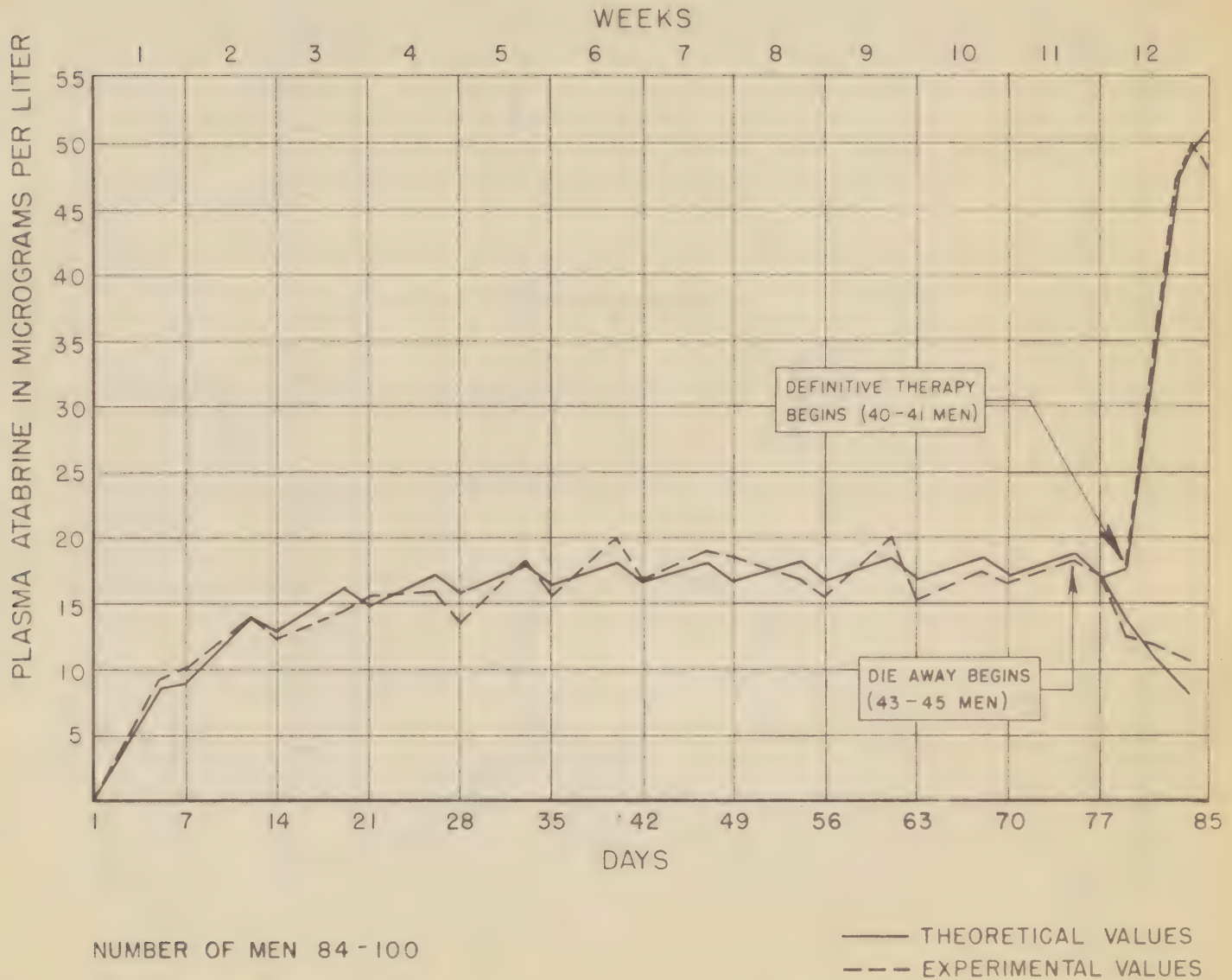


CHART —5

COMPARISON OF THEORETICAL AND EXPERIMENTAL PLASMA ATABRINE LEVELS 1.2 GM. THE FIRST WEEK AND 0.6 GM. EVERY SUBSEQUENT WEEK

EXPERIMENTAL CURVE BASED ON THE RESULTS FOR 30 MEN

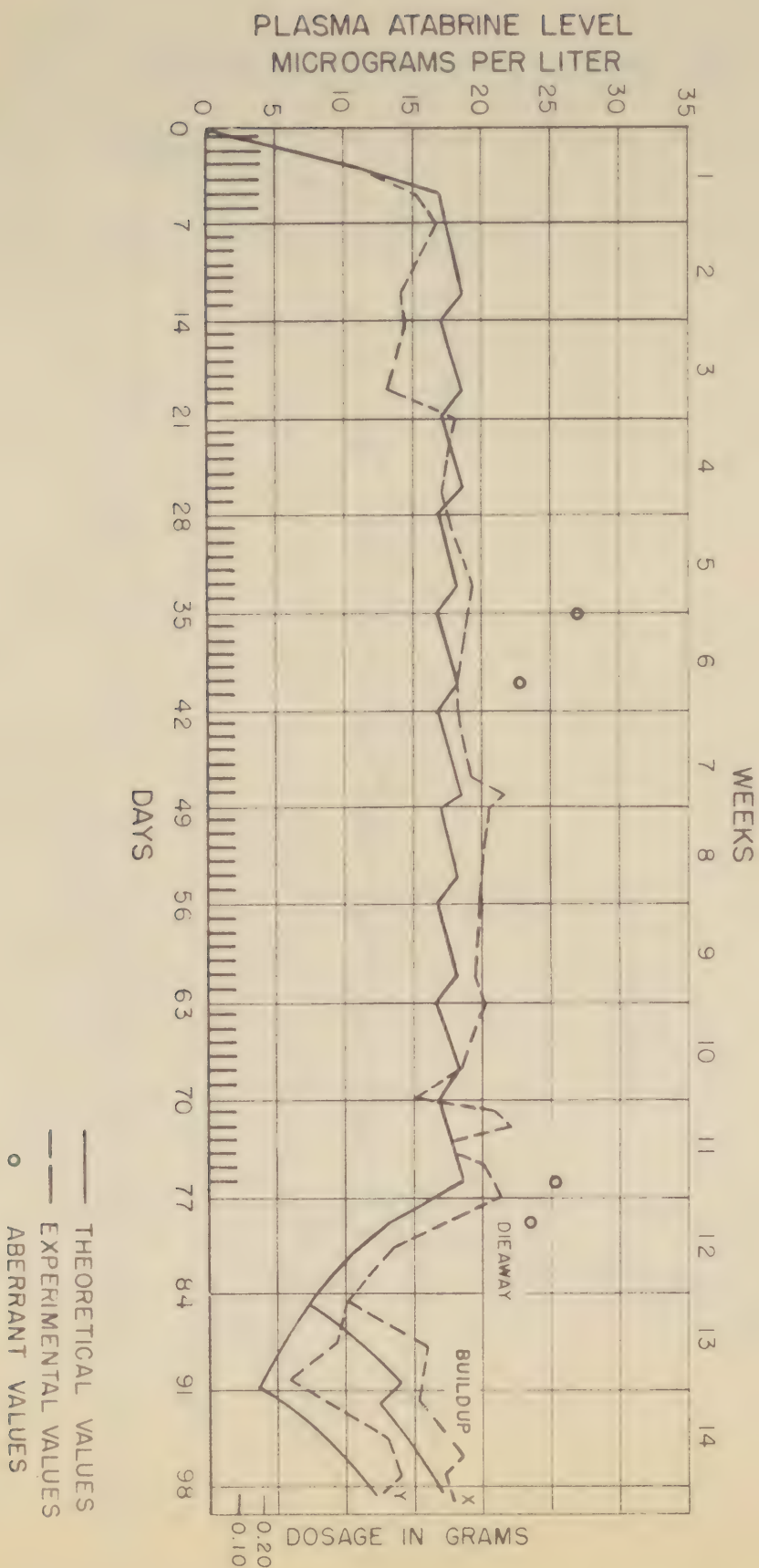


CHART - 6

THEORETICAL FLASMA ATABRINE LEVELS OF ALL MAJOR GROUPS FOR ENTIRE EXPERIMENT

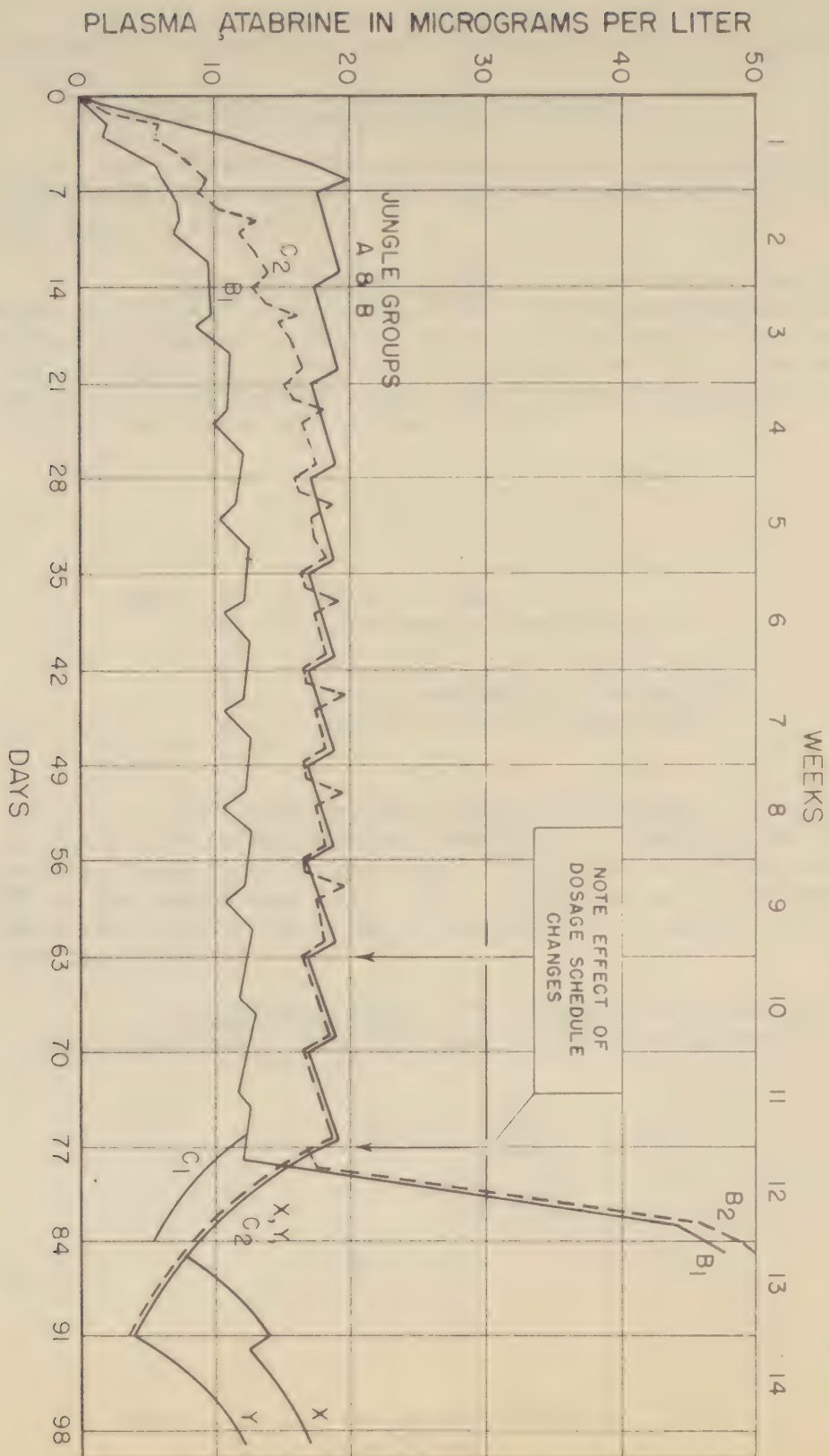


CHART - 7

CHART - 7

the greater loss arising from transient elevation of level.

5. Consequences of the simple mechanism.

a. Certain facts in regard to the course which a group will follow arise from the two relations postulated for our simple mechanisms (par. 3, (1) and (2)).

b. Rise in atabrine level. If atabrine is administered in equal daily doses, underlying level rises by diminishing increments to an equilibrium value, while loss per day becomes larger and larger, until at equilibrium the loss just equals the daily dose contribution. The rise is logarithmic, approaching the equilibrium value asymptotically. It may be expressed by the equation:

$$L = L_1 (1 - e^{-Kt})$$

Where L = level at time t .
 L_1 = equilibrium level ($t = \infty$).
 t = time after first dose. (days)
 K = decay constant in $1/\text{days} = 0.1$.
 e = Napierian base of logarithms.

c. This rise may be described as follows: ten percent of the remaining rise to equilibrium takes place in each day so that: 50% of the rise takes place in one week, 75% by the end of the second week, 87% by the end of the third, etc. By the end of the sixth week 98% has taken place and only 2% of the total rise remains. This is within experimental error.

d. Equilibrium level. The equilibrium value (L_1) is proportional to the dose. Thus, for equilibrium (gain = loss):

$$AD = K (L_1 + AD)$$

$$L_1 = AD \left(\frac{1}{K} - 1 \right)$$

Since,

$$K = 0.1$$

$$L_1 = 9 AD$$

Where A = rise per gm of dose.
 D = dose in gm.

From paragraph 3-(1) above, $A = 23.2$ micrograms/L/gm dose. Hence,

$$L_1 \text{ (in micrograms/L)} = 209 \times D \text{ (in gms.)}$$

This direct proportionality between the equilibrium concentration and daily dose is of the greatest practical importance since it provides the means for predicting the maintenance level achieved by any dosage regimen. Thus, for example, for 0.15 gm daily the underlying level will be:

$$209 \times 0.15 = 31.3 \text{ micrograms/L.}$$

While strictly true only for a regular daily regimen, the average equilibrium level can be determined for an irregular regimen, repeated weekly, with sufficient accuracy by -

$$L_1 = \frac{209}{7} \times D' = 30 \times D'$$

Where D' is the weekly dose in gm.

Hence, for a weekly dose of 0.6 gm the underlying level will be $30 \times 0.6 = 18$ micrograms/L.

e. Decay of level after termination of dosage. After cessation of atabrine administration the level will fall logarithmically:

$$L = L_0 e^{-Kt}$$

Where L = level at time t .
 L_0 = level at time $t = 0$.
 t = time in days after L_0 .
 K = decay constant in 1/days = 0.1.
 e = Napierian base of logarithms.

f. This decay is very similar to the rise since: - Ten percent of the level is lost each day so that; 50% will be lost after one week; 75% after two weeks, etc. At the end of six weeks only 2% of the equilibrium level will remain.

g. The general course - rise, equilibrium and decay - is illustrated in Chart 8 for a daily dose of 0.05 gm.

h. Mathematical derivation for continuous administration.
 Provided there is approximately continuous administration of the drug, these three relationships may be derived mathematically from the simple statement in paragraph 3 as follows -

$$dL = AD \, dt - KL \, dt$$

$$\text{or} \quad \frac{dL}{AD - KL} = dt$$

$$\text{by integration,} \quad -\frac{1}{K} \log (AD - KL) = t - \log C_1$$

Where dL = change in concentration in time increment, dt .

C_1 = constant of integration.

A, D, L and K as above.

$$-\frac{1}{K} \log_e \left(\frac{AD - KL}{C} \right) = t$$

$$\frac{AD - KL}{C} = e^{-Kt}$$

$$L = \frac{AD}{K} - \frac{C}{K} e^{-Kt}$$

$$\text{At } t = \infty, \quad L = L_1 = \frac{AD}{K}$$

$$\text{At } t = 0, L = 0, \text{ so, } \frac{C}{K} = \frac{AD}{K} \text{ and } C = AD$$

$$\text{Hence,} \quad L = L_1 (1 - e^{-Kt}) = AD (1 - e^{-Kt})$$

$$\text{and} \quad L_1 = \frac{23.2}{0.1} D \text{ or } 232 D$$

THEORETICAL CURVES

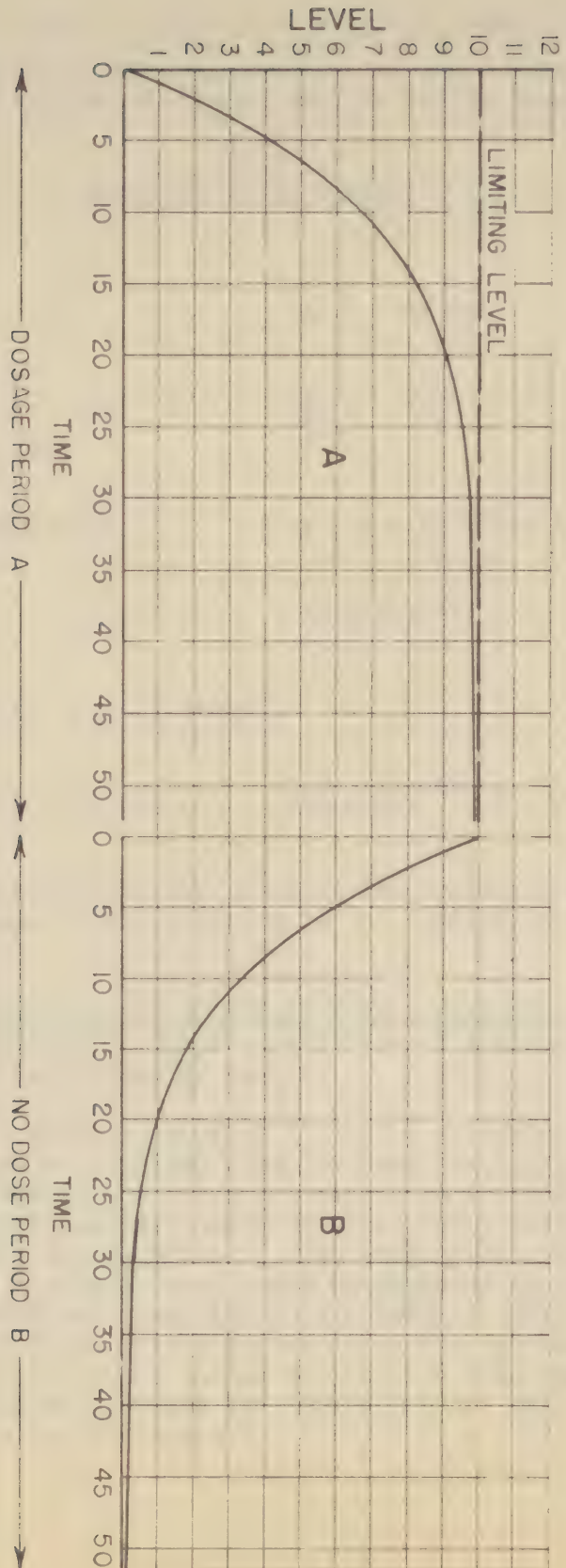


CHART - 8

The actual value $L_1 = 209$ instead of 232, results from the discontinuous administration of the drug and the procedure developed to deal with it, in contrast to the continuous administration assumed in the foregoing analysis.

i. In a period of no dosage, $D = 0$, so from above differential equation:

$$dL = -KL \, dt$$

$$\frac{dL}{L} = -K \, dt$$

by integration, $\text{Log } L = -Kt + \log C$

$$L = C e^{-Kt}$$

At $t = 0$, $L = L_0$, hence

$$L = L_0 e^{-Kt}$$

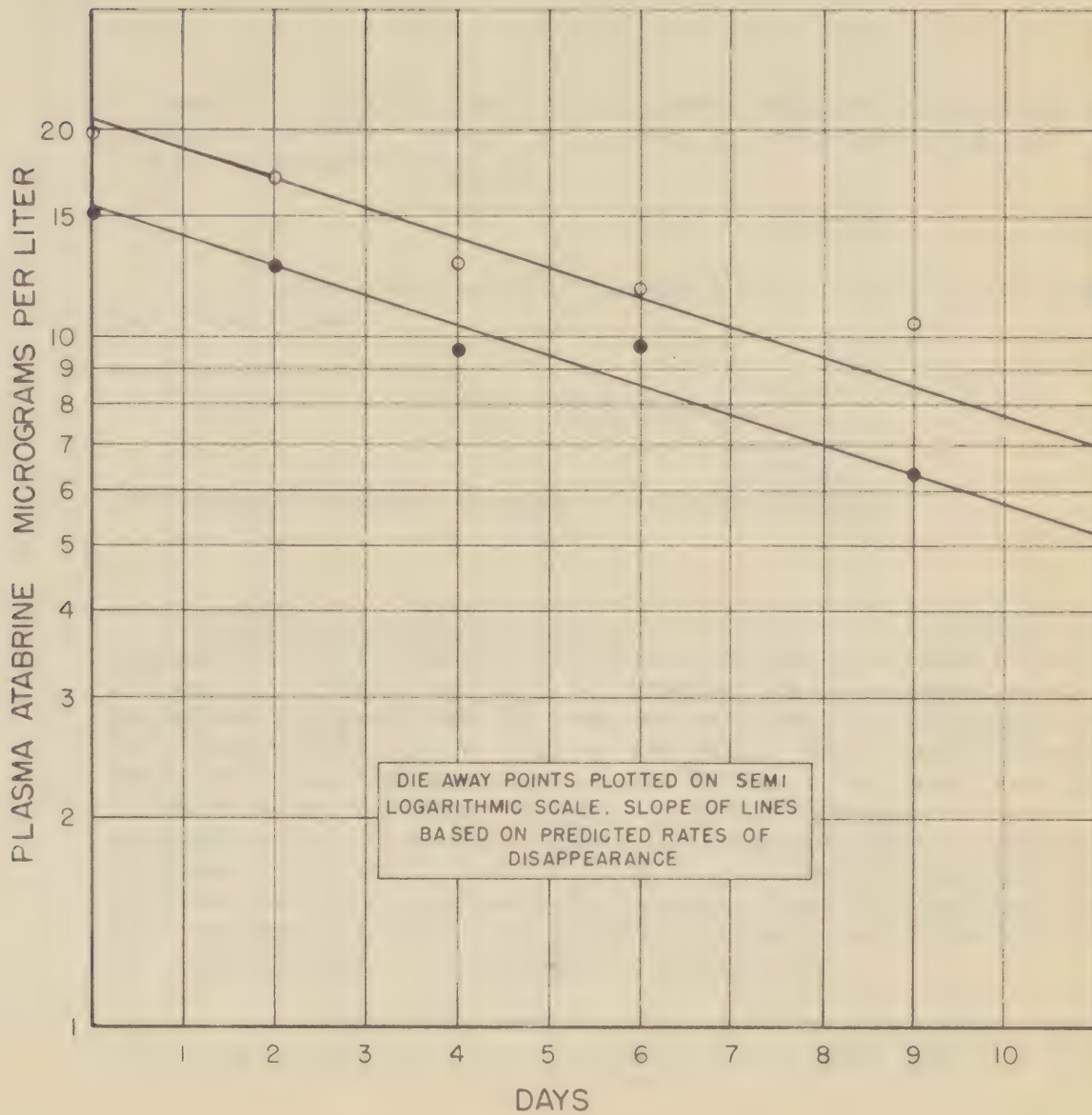
Where L_0 is the value at the beginning of decay or termination of dosage. This equation is the expression for dieaway given in paragraph 5-e.

6. Evaluation of constants from experimental data. The values of the constants for the two relations of the simple mechanism were obtained in the following manner:

a. Determination of K . It is evident that K , the decay constant, can be obtained from the experimental data, both from the rising portion of the curve and also from the decay. Since none of the regimens followed, comprised equal daily doses but rather a weekly repetition of dosage pattern, only those plasma concentrations obtained at the same time each week could be employed for determination of K . The value of K was obtained by adjustment to give the best fit on the rising portion of the curves drawn through these corresponding points. The resulting values may be then compared with the observed decay, See Chart 9, where the points are the actual observed values and the line has the slope K .

CHART -9

EXPONENTIAL NATURE OF THE DISAPPEARANCE
OF ATABRINE FROM THE PLASMA
WHEN DOSAGE IS DISCONTINUED



COMPANY C, SECTION 2 ○ (0.6 GM GROUP)
COMPANY C, SECTION 1 ● (0.4 GM GROUP)

b. Determination of A. Knowing the values of K and the equilibrium level, L_1 , for a given dosage rate, D, the value of A, (rise per 1 gm dose) may then be obtained by the relation already given in paragraph 5-d:

$$L_1 = AD \left(\frac{1}{K} - 1 \right) \text{ or}$$

$$A = \frac{L_1}{D \left(\frac{1}{K} - 1 \right)}$$

Values of the equilibrium level (L_1) were obtained for each set of group data and the average daily dose, D, determined (e.g., for the 0.6 gm/wk groups $D = \frac{0.6 \text{ gm}}{7}$).

c. The best value for the equilibrium levels of all groups was found to be:

$$L_1 = 209 \text{ D micrograms/L.}$$

from which we obtain $A = \frac{209}{9} = 23.2 \text{ micrograms/L.}$

7. Evaluation of Transients. While the data on changes in plasma concentration which immediately follow the administration of atabrine are meager, a tentative basis for computation is suggested which has satisfactorily predicted the response obtained from therapeutic regimens. In a considerable number of cases two maxima occur, one at about 2 hours after dose and another at about 8 hours after dose (see Chart 10). In other cases no bimodal form is noticeable. However, it is a common observation that bimodality may be lost sight of, if the time spacing is somewhat variable. The fact that it does occur frequently is strong evidence that a dual mechanism is present. We shall therefore assume that two distinct transients occur but are so closely spaced that they may merge at times. Our concept is as follows:

a. A first transient (T_1 , Charts 11 and 12) begins immediately after administration, rises to a maximum in 2 hours and falls to a negligible value at the end of 8 hours.

b. A second transient (T_2 , Charts 11 and 12) follows more gradually after administration, rises to a maximum in 8 hours and then decays, losing a half value in each succeeding four-hour interval. Thus, it has practically disappeared 24 hours after dosage.

CHART-10

POST ABSORPTION CURVES OF PLASMA ATABRINE CONCENTRATION
FOLLOWING 0.2 GM DOSE

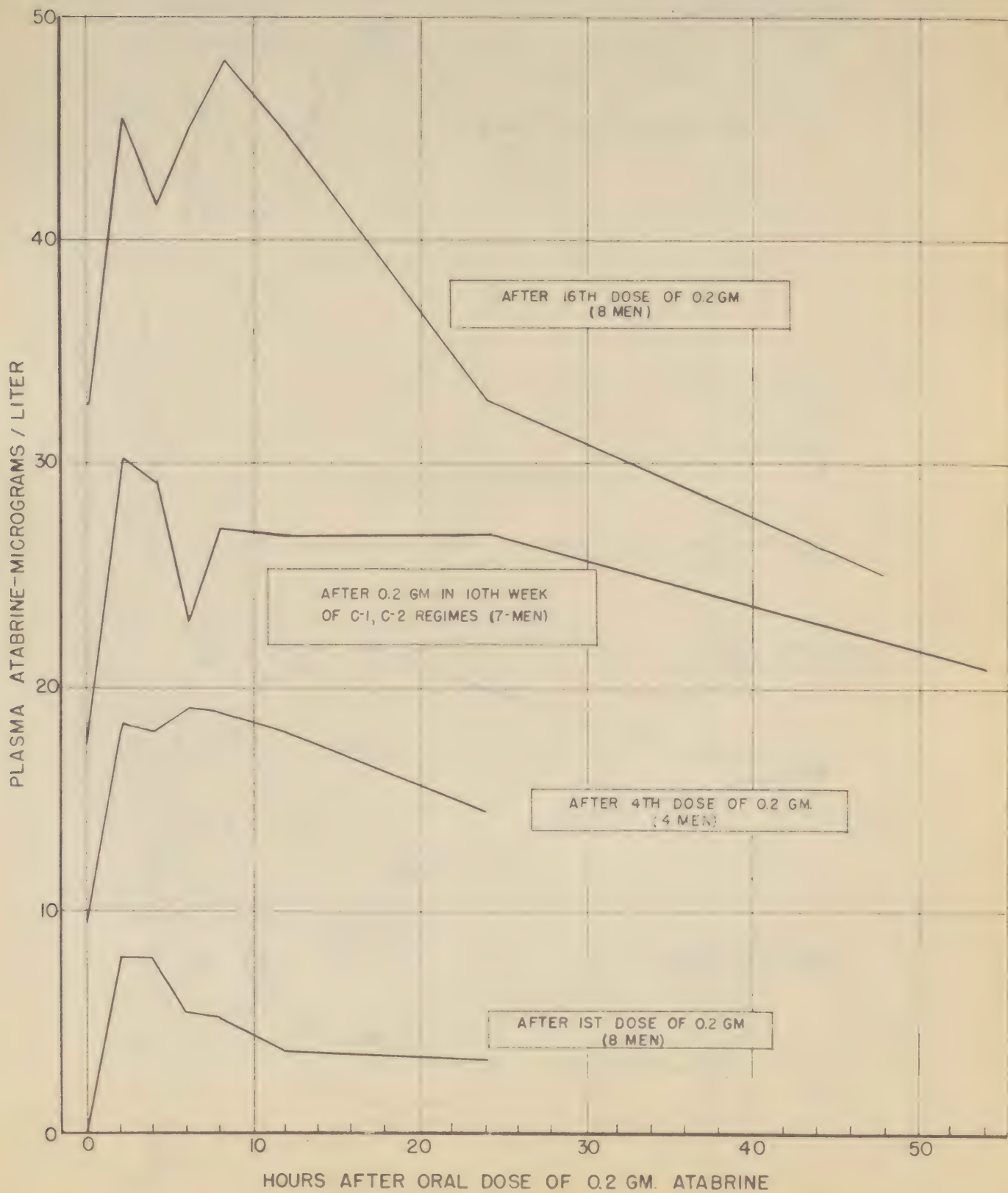


CHART-10

CHART - II

TRANSIENTS FOLLOWING SINGLE DOSE 0.2 GM.

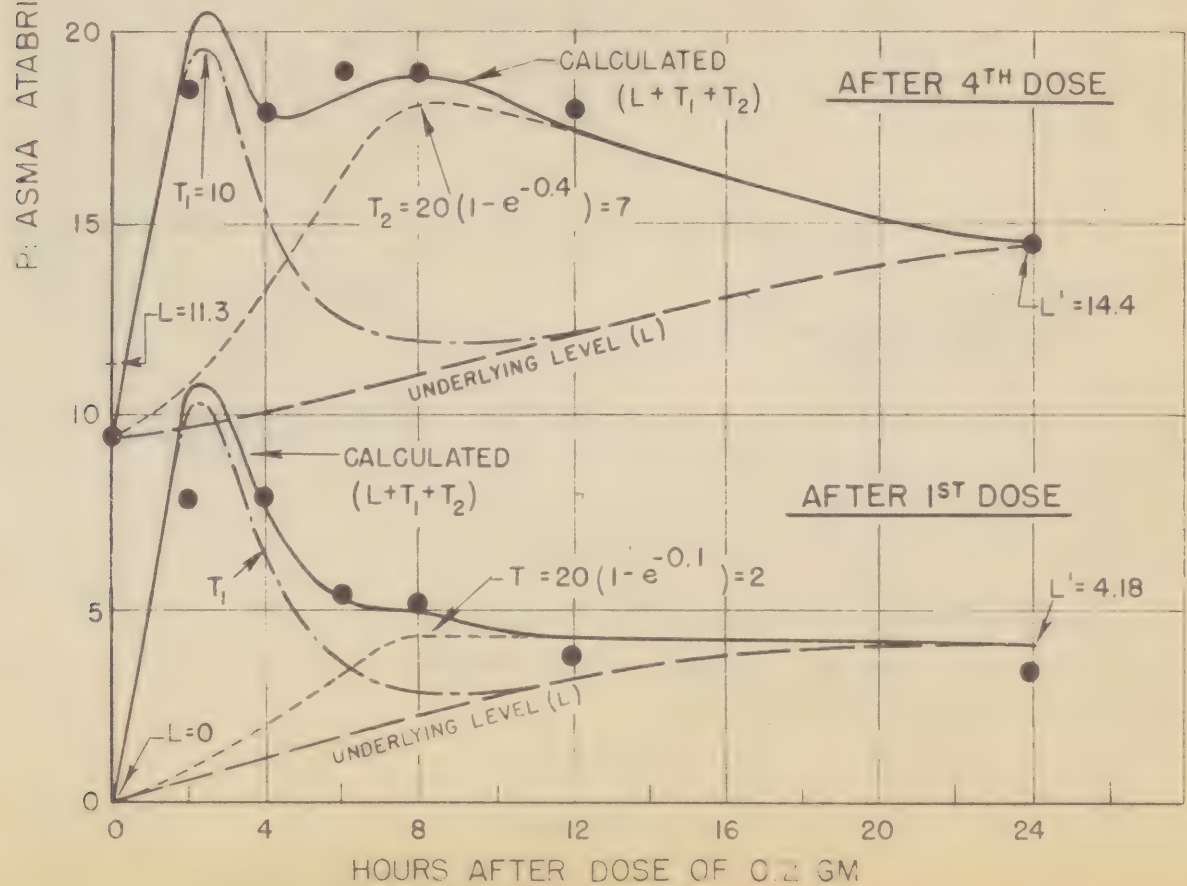
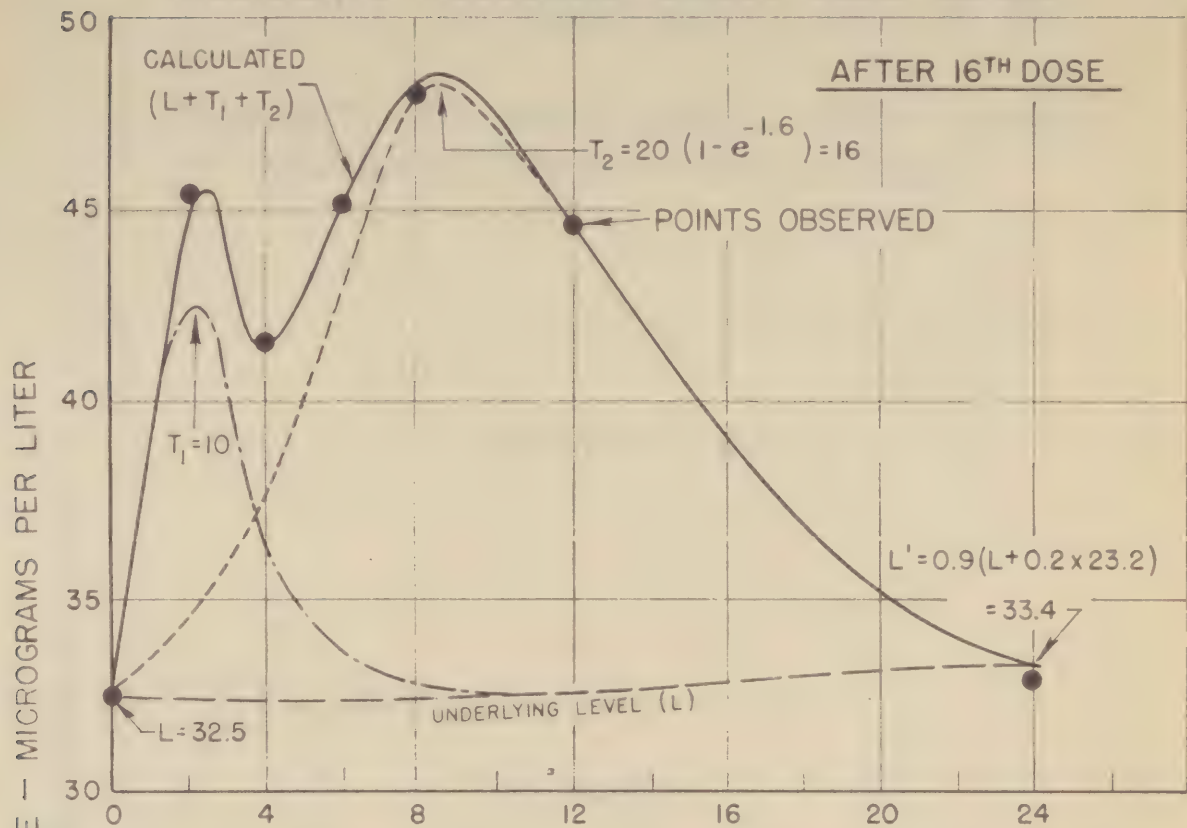
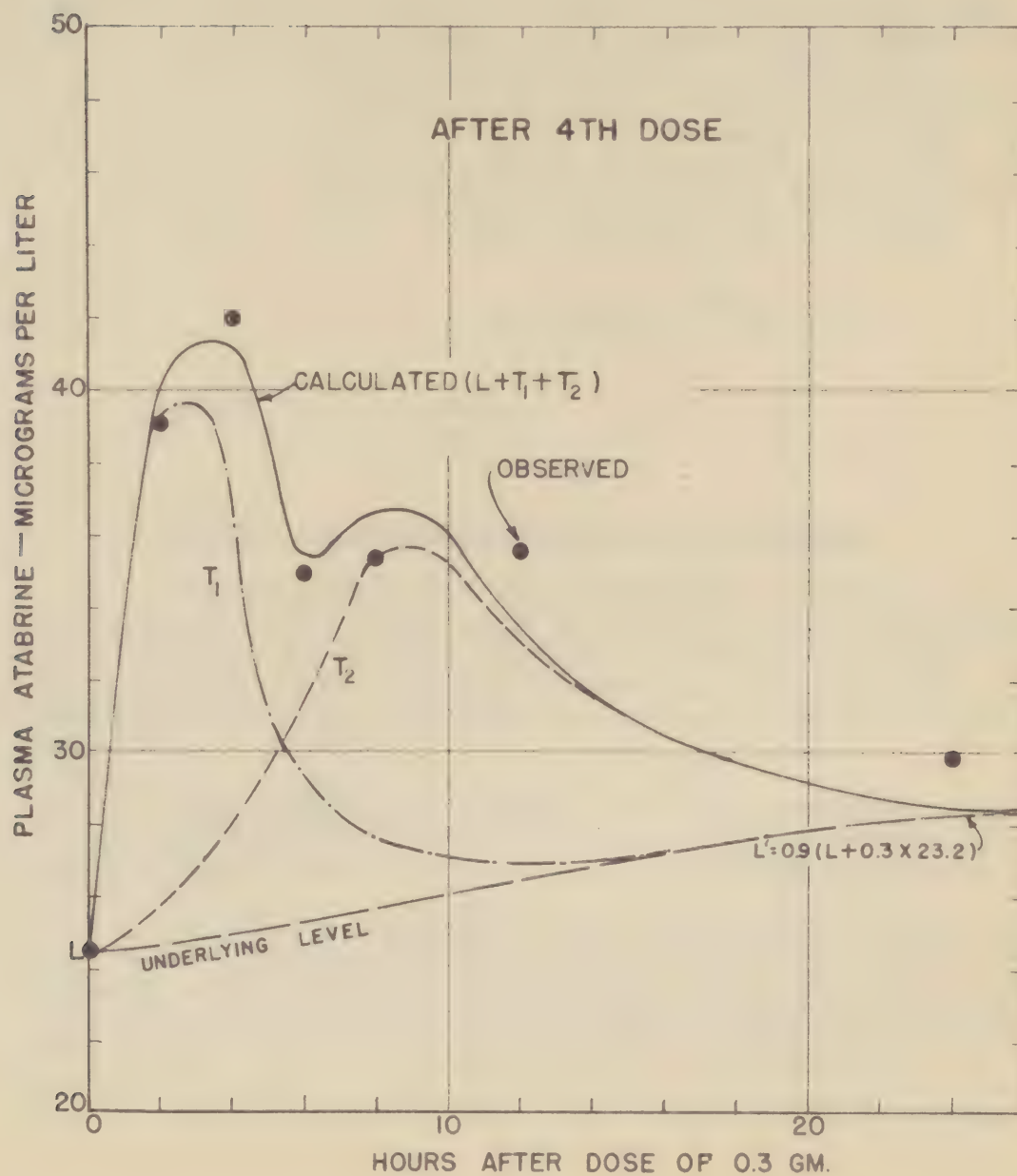


CHART - II

CHART-12

TRANSIENTS FOLLOWING SINGLE DOSE 0.3 GM.
CALCULATED CURVES—OBSERVED POINTS



c. The contribution of these two transients is additive and superimposed on the underlying level which undergoes a gradual change from the value of one day to that of the next.

d. The magnitudes of the peaks of these two transients were found to be approximately as follows:

- (1) The two hour transient is proportional to the dose and equals 50 micrograms/L/gm dose.
- (2) The 8 hour transient varies from a small peak value at the beginning of treatment to a maximum peak value when the subject has attained equilibrium on that particular dosage regimen. This greatest value is found to be 100 micrograms/gm dose. The increase in peak magnitude in the second transient is similar to the logarithmic rise of the underlying level. It may be roughly expressed by the expression:

$$T_2 = 100 (1 - e^{-Kt}) D$$

Where $K = 0.1$ as before.

t = day of dose.

8. Application of theory to therapeutic regimens.

The underlying level for a therapeutic regimen may be computed in the manner already described. However the doses are administered so frequently that a continuous curve may be drawn by consideration of the exponential form. Superimposed upon this underlying curve the contributions of each of both transients from each dose may be determined graphically as in Chart 13.

9. Physiological implications. While the preceding correlation of the observed data has been presented on a purely empirical basis, possible implications as to physiological mechanism are of some interest.

a. The first transient. The fact that the magnitude depends only on the particular dose causing it and not on the stage of the treatment or the underlying level suggests a direct effect of absorption. The relatively short time required to reach maximum (2 hours) and the brief overall duration is compatible with this interpretation.

b. The second transient. The marked variation in peak

THEORETICAL RESPONSE TO THERAPEUTIC REGIMEN

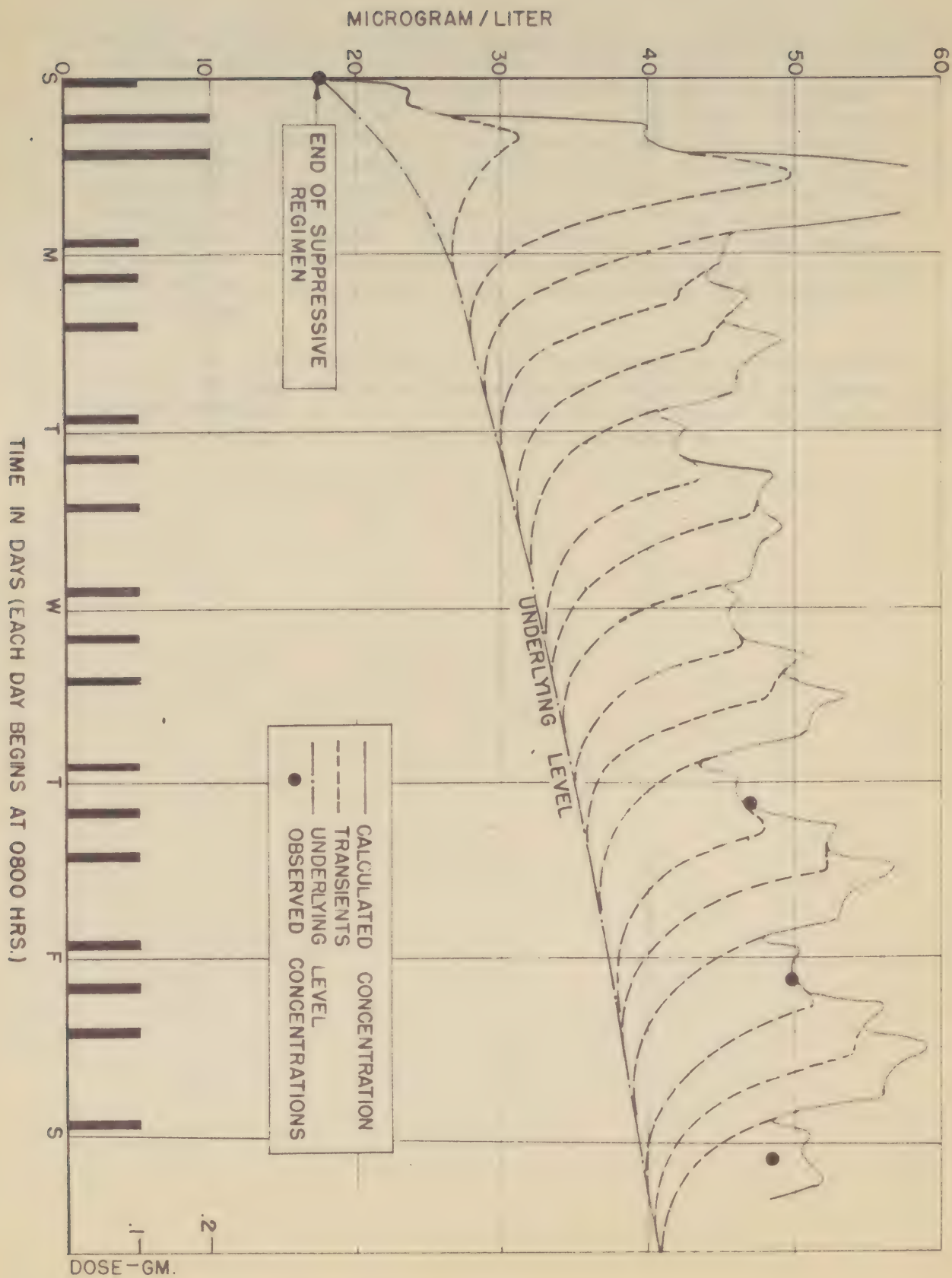


CHART-13

magnitude of this transient is suggestive of a contribution arising after passage thru a barrier in which partial removal has taken place. This barrier at first passes only a small fraction of what later passes when saturation of the barrier has taken place. The greater time between administration of dose and maximum of the transient, is again suggestive of passage through a barrier. The nature of the progressive rise in magnitude of this transient suggests that the saturation of the barrier follows a parallel course to the general saturation of the system. The time of decay of this transient is suggestive of a redistribution rather than elimination from the body which follows a much slower course. The liver has been suggested as this barrier.

c. The underlying level may be thought of as the concentration in the blood plasma maintained by the buffering of the systemically stored atabrine. It is to be expected that the values relating to this systemic storage would present the simplest picture.

Run No.	Time hr.	LEVEL mm Hg	DOSE mg
0	27	0	50
1	28	1.04	50
2	29	1.98	0
3	30	1.78	100
4	31	3.79	100
5	31.5	3.50	50
6	32	6.00	50
7	33	6.44	50
8	34	6.94	50
9	35	7.29	0
10	36	6.56	100
11	37	7.99	100
12	38	9.28	50
13	39	9.40	50
14	40	9.50	50
15	41	9.59	50
16	42	9.68	0
17	43	8.71	100
18	44	9.93	100
19	45	11.02	50
20	46	10.96	50
21	47	10.77	50
22	48	10.33	50
23	49	10.79	0
24	50	9.71	100
25	51	10.83	10
26	52	11.84	50
27	53	11.70	50
28	54	11.57	50
29	55	11.46	50
30	56	11.36	0
31	57	10.22	100

DAY of EXPER	DATE	LEVEL TELORIT	DOSE mg
32	27	11.29	100
33	29	11.25	50
34	30	12.07	5
35	Oct 1	11.92	50
36	2	11.75	50
37	3	11.2	0
38	4	15.46	100
39	5	11.50	100
40	6	12.44	50
41	7	12.24	50
42	8	12.06	50
43	9	11.90	50
44	10	11.75	0
45	11	10.57	100
46	12	11.60	100
47	13	12.53	50
48	14	11.32	50
49	15	12.13	50
50	16	11.96	50
51	17	11.81	0
52	18	10.63	100
53	19	11.65	100
54	20	12.57	50
55	21	12.36	50
56	22	12.17	50
57	23	12.00	50
58	24	11.84	0
59	25	10.66	100
60	26	11.68	100
61	27	12.60	50
62	28	12.38	50
63	29	12.19	50

[illegible]

THEORETICAL PLASMA ATABrine
LEVELS ON SUPPRESSIVE DOSE

400 mg per week

COMPANY B SECTION 1.

Dosage Constant - 2.32 micrograms/liter/ 100 mg. dose
Die-Away Constant - 0.10.

Underlined Levels occur on days on which men were bled.

DAY of EXPER.	DATE	PLASMA-DOSE LEVEL THEORET.	DOSE mg.
0	AUG. 27	0	100
1	28	2.09	200
2	29	6.06	0
3	30	5.45	100
4	31	6.99	100
5	Oct. 1	<u>8.33</u>	100
6	2	9.63	0
7	3	<u>8.67</u>	100
8	4	9.81	200
9	5	13.07	0
10	6	<u>11.76</u>	100
11	7	<u>13.67</u>	100
12	8	<u>13.49</u>	100
13	9	<u>14.23</u>	0
14	10	<u>12.81</u>	100
15	11	<u>13.62</u>	200
16	12	16.44	0
17	13	14.80	100
18	14	15.41	100
19	15	<u>15.96</u>	100
20	16	16.45	0
21	17	<u>14.81</u>	100
22	18	15.42	200
23	19	18.06	0
24	20	16.26	100
25	21	16.72	100
26	22	<u>17.14</u>	100
27	23	17.52	0
28	24	<u>15.77</u>	100
29	25	16.28	200
30	26	18.32	0
31	27	16.94	100

DAY of EXPER.	DATE	PLASMA-DOSE LEVEL THEORET.	DOSE mg.
32	28	17.34	100
33	29	17.69	100
34	30	18.01	0
35	Oct. 1	<u>16.21</u>	100
36	2	16.68	200
37	3	19.19	0
38	4	17.27	100
39	5	17.63	100
40	6	<u>17.95</u>	100
41	7	18.24	0
42	8	<u>18.42</u>	100
43	9	16.87	200
44	10	19.36	0
45	11	17.42	100
46	12	17.77	100
47	13	<u>18.09</u>	100
48	14	18.37	0
49	15	<u>16.53</u>	100
50	16	16.96	200
51	17	19.44	0
52	18	17.50	100
53	19	17.82	100
54	20	<u>18.13</u>	100
55	21	18.40	0
56	22	<u>16.56</u>	100
57	23	16.99	200
58	24	19.47	0
59	25	<u>17.52</u>	100
60	26	17.85	100
61	27	<u>18.15</u>	100
62	28	18.42	0
63	29	<u>16.58</u>	100

DAY of EXPER.	DATE	PLASMA-DOSE LEVEL THEORET.	DOSE mg.
64	30	17.01	100
65	31	17.40	100
66	Nov. 1	17.35	100
67	2	17.15	100
68	3	<u>18.42</u>	100
69	4	18.64	0
70	5	<u>16.80</u>	100
71	6	17.21	100
72	7	17.56	100
73	8	17.89	100
74	9	18.19	100
75	10	<u>18.46</u>	100
76	11	18.70	0
77	12	<u>16.83</u>	100

THEORETICAL PLASMA ATABINE
LEVELS ON SUPPRESSIVE DOSAGE

600 mg. per week

COMPANY B SECTION 2.

Dosage Constant - 2.32 micrograms/liter/100 mg. dose
Die-Away Constant - 0.10.

Underlined Levels occur on days on which men were bled.

DAY of EXPER.	DATE	LEV-1 TH. RES.	DOSE mg.
0	Aug. 9	0	200
1	10	4.13	200
2	11	7.94	200
3	12	<u>11.32</u>	200
4	13	14.36	200
5	14	<u>17.10</u>	200
6	15	19.57	0
7	16	<u>17.61</u>	100
8	17	17.93	100
9	18	18.22	100
10	19	18.49	100
11	20	18.73	100
12	21	<u>18.94</u>	100
13	22	19.13	0
14	23	<u>17.22</u>	0
15	24	17.59	100
16	25	17.92	100
17	26	<u>17.22</u>	100
18	27	18.49	100
19	28	<u>12.72</u>	100
20	29	18.94	0
21	30	<u>17.05</u>	100
22	31	17.43	100
23	Sept. 1	17.78	100
24	2	18.09	100
25	3	18.37	100
26	4	<u>18.62</u>	100
27	5	18.85	0
28	6	<u>16.96</u>	100
29	7	17.35	100
30	8	17.71	100
31	9	18.03	100

DAY of EXPER.	DATE	LEV-1 TH. RES.	DOSE mg.
32	10	18.31	100
33	11	<u>18.57</u>	100
34	12	18.80	0
35	13	<u>16.91</u>	100
36	14	17.31	100
37	15	16.67	100
38	16	17.92	100
39	17	18.28	100
40	18	<u>18.52</u>	100
41	19	18.76	0
42	20	<u>16.83</u>	100
43	21	17.28	100
44	22	17.64	100
45	23	17.96	100
46	24	18.25	100
47	25	<u>18.51</u>	100
48	26	<u>18.15</u>	0
49	27	<u>16.87</u>	100
50	28	17.24	100
51	29	17.63	100
52	30	17.95	100
53	Oct. 1	18.24	100
54	2	<u>12.50</u>	100
55	3	18.74	0
56	4	<u>16.47</u>	100
57	5	17.27	100
58	6	17.63	100
59	7	19.95	100
60	8	18.24	100
61	9	<u>12.50</u>	100
62	10	18.74	0
63	11	<u>16.87</u>	100

DAY of EXPER.	DATE	LEV-1 TH. RES.	DOSE mg.
64	12	17.27	100
65	13	17.63	100
66	14	17.95	100
67	15	18.24	100
68	16	<u>18.50</u>	100
69	17	<u>18.74</u>	0
70	18	<u>16.87</u>	100
71	19	<u>17.27</u>	100
72	20	<u>17.63</u>	100
73	21	17.95	100
74	22	18.24	100
75	23	<u>18.50</u>	100
76	24	<u>18.74</u>	0
77	25	<u>16.47</u>	0
78	26	15.00	0
79	27	<u>12.50</u>	0
80	28	12.15	0
81	29	<u>10.93</u>	0
82	30	9.84	0
83	31	8.36	0
84	Nov. 1	<u>7.97</u>	0
85	2	7.71	0
86	3	6.45	0
87	4	5.80	0
88	5	5.22	0
89	6	4.70	0
90	7	4.23	0
91	8	3.81	100
92	9	5.52	100
93	10	7.06	100
94	11	8.44	100
95	12	9.68	100
96	13	10.80	100
97	14	11.81	-

THEORETICAL PLASMA LEVELS OF JUNGLE GROUPS
COMBINED A AND B GROUPS

1200 mg. dosage in first 6 days, 600 mg. per week thereafter.
Dosage Constant - 2.32 micrograms per liter per 100 mg. dose.
Die-Away Constant- 0.10.
Underlined Levels occur on days on which men were bled.



COMPANY C SECTION 1

DAY OF EXPER	DATE	PRE-DOSE LEVEL THEORET. (mg.)	DOSE
71	Nov 7	<u>11.87</u>	50
72	8	11.73	50
73	9	<u>11.60</u>	100
74	10	12.53	50
75	11	12.32	50
76	12	12.13	0
77	13	<u>10.92</u>	0
78	14	9.91	0
79	15	<u>8.92</u>	0
80	16	8.03	0
81	17	<u>7.23</u>	0
82	18	6.51	0
83	19	5.86	0
84	20	<u>5.26</u>	0

COMPANY C SECTION 2

DAY OF EXPER	DATE	PRE-DOSE LEVEL THEORET. (mg.)	DOSE
71	Nov 7	17.21	100
72	8	17.56	100
73	9	17.89	100
74	10	18.19	100
75	11	<u>19.46</u>	100
76	12	18.70	0
77	13	<u>16.83</u>	0
78	14	15.15	0
79	15	<u>13.64</u>	0
80	16	12.28	0
81	17	<u>11.05</u>	0
82	18	9.95	0
83	19	8.96	0
84	20	<u>8.06</u>	0

PLASMA ATABRINE LEVELS

THEORETICAL VALUES FOR DISAWAY AFTER SUPPRESSIVE THERAPY

Dosage Constant - 2.32 micrograms/liter/100 mg. dose.

Disaway Constant - 0.10

Underlined Levels occur on day on which men were bled.

APPENDIX C

PROCEDURES AND RESULTS OF INDIVIDUAL STUDIES

SECTION I - Collection, Preparation and Analysis of Plasma.

1. Principles of analysis.

a. The procedure used for the analysis of plasma for atabrine was that developed by Brodie and Shannon (Malaria Report No. 9, N.R.C.). Briefly, the method is based on measurement of the intensity of fluorescence, which is proportional to the concentration of atabrine in a suitable medium. Preliminary removal of interfering fluorescent materials is accomplished in two steps; (1) by extraction of the organic base, atabrine, from alkalinized plasma by shaking with ethylene dichloride, (2) transfer of the atabrine to concentrated lactic acid, again by shaking, from the ethylene dichloride. The fluorescence is read directly in the lactic acid solution with a photoelectric fluorometer. Minor modifications were made to facilitate handling large numbers of specimens.

b. Atabrine is present in the leukocytes in very much higher concentration than in plasma. Since leukocytes begin to disintegrate shortly after blood is drawn, it is imperative to separate the plasma soon after drawing the blood in order to avoid contamination of the plasma by leukocyte atabrine. It is necessary to free the plasma of all intact leukocytes by adequate centrifugation. These conditions can be fulfilled only when the blood stands less than 15 minutes before centrifugation. The possibility of accidental contamination by leukocytes is minimized by the removal of plasma after 15 minutes of centrifugation after which the plasma is spun an additional hour. Considerations of speed in handling the blood as well as the desirability of reducing interference with military training to a minimum, led to the final procedure for obtaining and processing the blood samples.

2. Collection of samples.

a. The bleeding schedules were arranged to collect 20 samples every 10 minutes (30 ml each). This number made for efficient use of the centrifuge equipment; each group of 20 specimens was treated as a unit, no additional samples being collected until these had been placed in the centrifuge. In this way flexibility of schedules was secured which permitted minor delays without endangering a large number of specimens. The ten-minute deadline insured ample leeway for meeting the maximum intervals of 15 minutes between withdrawal of blood and start of centrifuging.

b. Three bleeding stations were required to secure the 20 specimens in this time. This required a maximum of 7 bleedings at each station in 10 minutes. This rate was easily managed by the team of assistant and bleeder manning each station. Generally the 20 samples were collected within 7 minutes; a complete section of 100 men was handled in 50 minutes. The blood was drawn in a 30 ml syringe, containing 6 drops of saturated potassium oxalate.

3. Preparation of samples.

a. The 6 or 7 samples collected at each bleeding station during one bleeding period were delivered to the preparation room in the small rack used to hold the tubes during the bleeding. The tubes were here transferred to the centrifuge for the first centrifugation (15 minutes running time, 3 minutes for deceleration). Thereupon the specimen tubes were removed and placed in a rack already half filled with numbered conical centrifuge tubes. The plasma was carefully aspirated with a clean dry 10 ml syringe fitted with a 3-inch No. 19 gauge needle and transferred into the conical tube. The schedule permitted 15 minutes for the transfer by 2 men of the 20 specimens in each run. At the completion of the first separation the plasmas were returned for the final 1-hour centrifugation.

b. When the centrifugation was finished a measured portion of the plasma was withdrawn from each conical tube without disturbing the sediment, and placed in a correspondingly numbered 60 ml bottle in a rack. The final aspiration was accomplished with a syringe pipette (Chart 14) calibrated to deliver 10 ml at full extension. The pipette was also graduated at full ml intervals down to 6 ml for use when less than the full amount of plasma was available. The syringe used to transfer plasma was rinsed 3 times with saline after each use. At the completion of the transfer a check was made of the plasma samples against the bleeding forms and the numbers were transferred to the analysis form. The samples (in dust-proof boxes) were held in a refrigerator at 40°F for analysis the next day.

4. Treatment of samples.

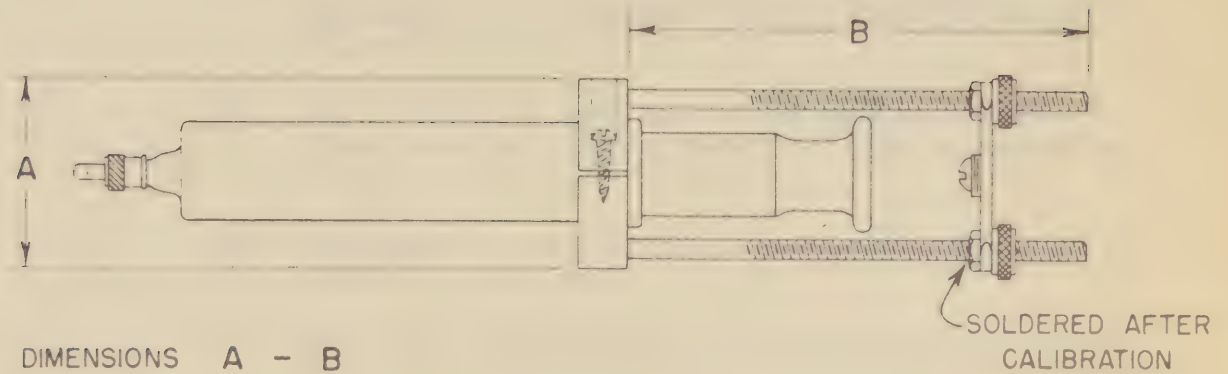
a. The plasma samples in the 60 ml glass-stoppered bottles were delivered to the laboratory in dust-proof boxes and rechecked against the analysis forms.

b. All samples for one day (up to 150 specimens and 25 controls and standards) was carried through as a unit. The use of numbered glassware for the various steps through which the material passed and the use of racks which had uniform spacing expedited the handling of the large numbers of specimens.

c. Each specimen and control recovery (on blood-bank plasma)

CHART -14

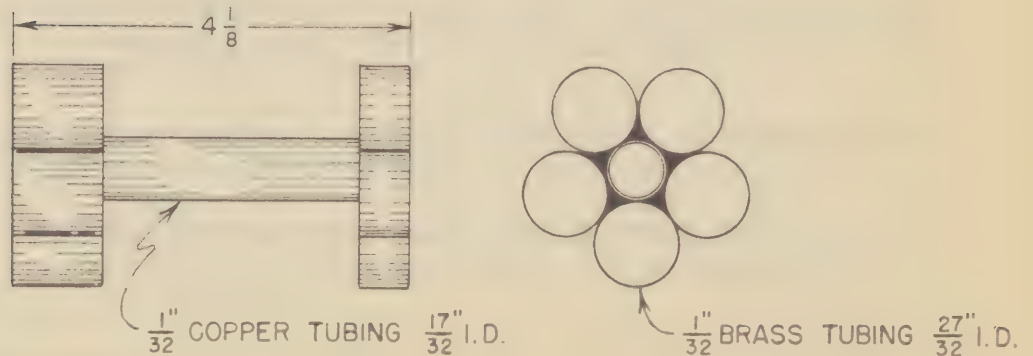
SYRINGE PIPETTE



DIMENSIONS A - B

10 ML.....	$\frac{9}{16}$	$4\frac{3}{8}$
30 ML.....	$\frac{7}{8}$	$4\frac{5}{8}$

INSERT TO ADAPT 250 ML TRUNNION CUPS TO CARRY 5 TEST TUBES



passed through the following steps, in order:

- (1) 3 ml of 0.2M Na_2HPO_4 is added to each bottle from an automatic 50 ml burette.
- (2) 30 ml of ethylene dichloride is added to each bottle from a calibrated syringe pipette.
- (3) 3 racks, containing 10 samples each, are shaken for 10 minutes in a mechanical shaker (240 cycles per minute).
- (4) The contents of each bottle are then poured into a 40 ml heavy duty pyrex centrifuge tube supported in a rack.
- (5) The tubes are centrifuged in a 16-place head, for 10 minutes at 2000 rpm.
- (6) The supernatant plasma is aspirated and discarded by means of a drawn glass tube connected to a water pump.
- (7) A 20 ml aliquot of ethylene dichloride is drawn off with a calibrated syringe pipette. The syringe is rinsed with ethylene dichloride three times between samples.
- (8) The aliquots are transferred to a second set of numbered 60 ml pyrex bottles.
- (9) 11 ml of diluted lactic acid (9 parts purified commercial lactic acid to 1 part distilled water) is added to each sample from a calibrated syringe pipette.
- (10) The bottles are shaken again in groups of thirty for 10 minutes.
- (11) After shaking, the samples are poured into numbered cuvettes (preselected 20 x 150 mm pyrex test tubes) in a wire rack.
- (12) The cuvettes are centrifuged for 10 minutes at 2000 rpm in a 16-place head.
- (13) The cuvettes are returned to the wire racks and placed in a water bath ready for reading in the fluorometer.

5. Measurement of fluorescence.

- a. A Colman fluorometer (Model 12) was modified to permit the

measurement of fluorescence to be made on the upper part of the cuvette. By this means it was possible to read the fluorescence of the lactic acid solution stratified above the ethylene dichloride, thus eliminating transfer to another tube.

b. Standard Pyrex test tubes (20 x 150 mm) were selected for cuvettes on the basis of uniformity of size and freedom from optical defects. A standard atabrine solution was read in each tube and only those tubes which gave readings within $\pm 1\%$ were selected.

c. The fluorometer was warmed for one hour before any measurements were made, thus increasing stability of the instrument. The electrical zero of the instrument was set with the shutter closed. The sensitivity was then set near its maximum, using a lactic acid atabrine standard equivalent to 100 micrograms of atabrine per liter (0.667 micrograms of atabrine base in 11 ml of diluted lactic acid). The sensitivity was checked by measuring the fluorescence of a standard every 5 or 10 readings, and readjusting to the original setting, if necessary. If the change was greater than 2 scale divisions, the tubes read in the meantime were retested.

d. The solutions and standards were kept in the same water bath for at least half an hour before reading to insure uniformity of temperature of all solutions. Readings were made as rapidly as possible to avoid temperature changes of the solutions while in the instrument.*

6. Calibration and calculation of atabrine concentration.

a. The primary standard used contained 85.0 mg of atabrine dihydrochloride dihydrate (corresponding to 66.67 mg of atabrine base) in a 1 liter of a M/15 phosphate buffer (pH 7.8). This solution was kept in the refrigerator and small samples withdrawn as needed.

b. A working standard was prepared daily by diluting 5 ml of the primary standard with 100 ml of phosphate buffer (pH 7.8) and distilled water to 500 ml. The final concentration of atabrine base was 667 micrograms/L. Intermediate standards varying from zero atabrine up to the concentration of the working standard were prepared by appropriate dilution of the working standard. The concentration of buffer was kept uniform in all dilutions.

c. The final standards used for the daily calibrations were prepared by shaking 1 ml of the diluted standard with 10 ml of lactic acid and 20 ml of ethylene dichloride. These were transferred to cuvettes and centrifuged in the same manner as described for the blood samples.

* The temperature coefficient of atabrine fluorescence in lactic acid was found to be approximately linear in the range from 10° to 50°C. It amounted to about 1.5 microgram/C° for a concentration equivalent to 100 microgram/L of plasma.

d. Permanent standards were made up in lactic acid essentially as described above for daily standards except that no ethylene dichloride was added. Cuvettes were filled with this solution and sealed. These standards were read every day to check the daily standards. Observations over a period of three weeks showed no appreciable change in the permanent standards.

e. Control recoveries were prepared by transferring 1.0 ml of each atabrine dilution to each of two 60 ml bottles containing 10 ml of human plasma which had been preserved in the frozen state. These solutions were then analyzed in the usual way.

f. The fluorometer readings were converted into plasma atabrine concentrations by means of a calibration curve which was determined for each day's readings. Values for both the standards and the recoveries were plotted and straight lines were fitted visually. During most of the period of study 6 standards were used (in duplicate) corresponding to plasma atabrine concentrations of 0, 12.5, 25, 50, 66, and 100 micrograms per liter and 6 recovery standards (in duplicate) on blood-bank plasma corresponding to 0, 8.3, 16.7, 33.3, 44.4 and 66.7 micrograms per liter.

g. The recovery calibration curve ordinarily corresponded to a recovery of 95% or better over most of the range. However, the zero atabrine plasma recovery value was generally slightly higher than the zero atabrine standard value, resulting in a crossing of the recovery and standard curves near the origin.

7. Washing of apparatus. All glassware was washed in hot calgonite solution, rinsed with hot tap water, then cold distilled water and dried in a drying oven.

8. Discussion of the method and the limits of its accuracy.

a. It is believed that the management of the bleeding and the plasma preparation were sufficiently controlled to meet all the requirements of the present study. Accidental contamination by leukocytes or extraneous fluorescent materials presented no problem. Out of several thousand analyses performed, there were no more than 8 or 10 instances in which contamination was demonstrated. A decided advantage is gained, of course, by the uniformity of handling which obtains when analyses are being carried out on a large scale.

b. Most of the plasma atabrine levels encountered were at the lower limits of suitability of the technic, as used. The limiting factors in the procedure are: (1) extraneous nonfluorescent light - this gave deflections equivalent to 8 to 12 micrograms per liter of atabrine; (2) extraneous fluorescence (irreducible blank in reagents) - amounting to another 8 to 12 micrograms per liter; (3) instrumental limitations, imposed by the sensitivity (always used at maximum sensitivity) of the instrument, stability of the instrument, stability

of the light source, and the residual inequality of the cuvettes. The net results of these factors was such that the reproducibility of results in the range up to 30 micrograms was generally within ± 2 micrograms. The absolute value of the analytical results was generally reliable enough to keep the overall precision within this range of ± 2 micrograms, though, in a few runs the calibration procedure undoubtedly led to considerably greater absolute errors.

c. The convention used in converting fluorometer readings to atabrine concentrations consisted in drawing the best straight line through the points fixed by the control recoveries on plasma. This procedure implicitly assumes that all plasma samples were subject to the same factors that influenced the control samples and that the control plasma recoveries in their significant characteristics were identical to the plasma samples that were analyzed. On a few runs the recoveries were considerably less than the usual 95%. In several of these cases it is believed that this procedure of assigning values based on the recoveries led to a correct readjustment of the analytical values; in several others, however, assignment of value on the basis of a low recovery line led to results which were inconsistent with the previous and subsequent behavior of the plasma levels of the men. In these cases the difficulty was not identified but the plasma used for the recoveries must remain suspect. In general, of course, the absolute values assigned in a comparative procedure such as used here can never be better than the values found for the comparison standards (in this case the control recoveries); hence, the inherent reproducibility of the procedure influences the absolute values and may lead to a systematic error in all determinations. An additional hazard which may lead to a small systematic error at low levels consists in unrecognized nonlinearity between galvanometer deflection and atabrine plasma level, the best line drawn through either recoveries or standards generally passed below the zero atabrine point. Presumably this resulted from nonlinear response of the instrument.

d. These pitfalls indicate the necessity of careful control of the procedure if useful results are to be secured at the lower plasma levels.

e. Certain fairly simple steps might permit more efficient and more reliable work in the very low ranges (up to 10 or 15 micrograms) of plasma atabrine. These are, (1) concentration of the same amount of atabrine into a smaller final volume (requiring modification of the fluorometer). This would lessen the difficulties due to low sensitivity of the instrument, the scattered light, and the blank fluorescence. (2) Improve the fluorometer as to stability and sensitivity. (3) Careful selection of filters to minimize the amount of exciting light passed to the photo-cell. (4) If possible, find a suitable substitute for lactic acid that can be freed more easily from fluorescent materials.

9. Reagents.

a. Lactic acid - Commercial lactic acid was tested by shaking with ethylene dichloride of known purity (as regards fluorescent materials) and reading in the fluorometer. If the blank value thus obtained was within one scale division of that given by purified (see below) lactic acid no purification was done. (1 scale division = 1.5 microgram per liter atabrine). Some lots of lactic acid have given slightly lower readings before purification than after, so it is regarded as desirable to omit purification unless there is definite evidence that it is necessary.

(1) Four-liter lots of lactic acid were shaken in a Pyrex glass stoppered bottle with about 4 grams of bone charcoal (Norit A Pfanstiehl) and allowed to stand overnight. The mixture was then treated with about 1.5 grams of Filter-Cel analytical grade (Johns Manville Co.) and filtered with suction through a 10 inch Büchner funnel, using two sheets of filter paper (Whatman #2). The filtrate in general gave a low blank, but since it usually gave a positive Tyndall effect it was refiltered through a 3-1/4 inch Pyrex sintred glass funnel, fine porosity. The filtrate thus obtained gave a negative or inappreciable Tyndall effect and a low blank. It was stored in Pyrex glass stoppered bottles and used without further purification.

b. Diluted lactic acid - 180 c.c. of distilled water is placed in 2 liter volumetric flask and made up to 2 liters with lactic acid.

c. Ethylene dichloride. In part of the work the ethylene dichloride was purified with Norit. Approximately 4 grams of Norit was added to ethylene dichloride in 4 liter bottles shaken, allowed to stand several hours, and filtered twice through filter paper. Extreme care must be taken to remove the last trace of charcoal.

d. Disodium phosphate solution. A 0.2 molar solution of Na_2HPO_4 .

e. Phosphate buffer solution was prepared by making a mixture of 9 parts of M/7.5 molar Na_2HPO_4 and 1 part of M/7.5 molar H_2PO_4 .

f. Stock standard - A sample of pure atabrine dihydrochloride dihydrate obtained from Dr. James A. Shannon's laboratory was used. On drying over sulfuric acid in a vacuum dessicator at room temperature the material lost 6% in weight, corresponding to 85% of the theoretical amount for 2 molecules of water of crystallization, and it also changed somewhat in color from canary yellow to orange yellow. 85.0 mg of the undried material was dissolved in water, 500 ml of M/7.5 buffer, pH7.8 (e, above) added and diluted to 1 liter with distilled water and stored in the refrigerator.

10. Personnel used.

a. Bleeding station at the Replacement Training Center.

- (1) Bleeders: - 3 officers.
- (2) Supervisor: 1 officer.
- (3) Assistant Bleeders: 2 (civilians) cleaned equipment in spare time and 1 (enlisted man) helped separate plasma at end of bleeding.
- (4) Plasma Preparation: 2 (enlisted men) sharpened and sterilized the needles, and arranged the equipment for the bleeding when not actually handling the samples.

b. Chemical Laboratory.

- (1) Technicians: (4 enlisted men) 3 of these worked on the actual analysis; one purified reagents, etc.
- (2) Supervisor: 1 officer.
- (3) Cleaning and preparation of glassware: 2 civilians.

11. Reliability of certain results.

Analytical difficulties with the specimens taken on the following dates gave basis for questioning the reliability of the values obtained: Jungle Groups, 9-11-43 (H₂ + 5 only) 9-13-43, 9-20-43, 10-27-43; Co. B, Section 1, 10-15-43, 10-18-43; Co. B, Section 2, 10-27-43; Co. C, Section 2, 9-18-43.

APPENDIX C

SECTION II - Plasma Atabrine Levels Established in Two Large Groups Under Different Dosage Regimens.

1. Procedure.

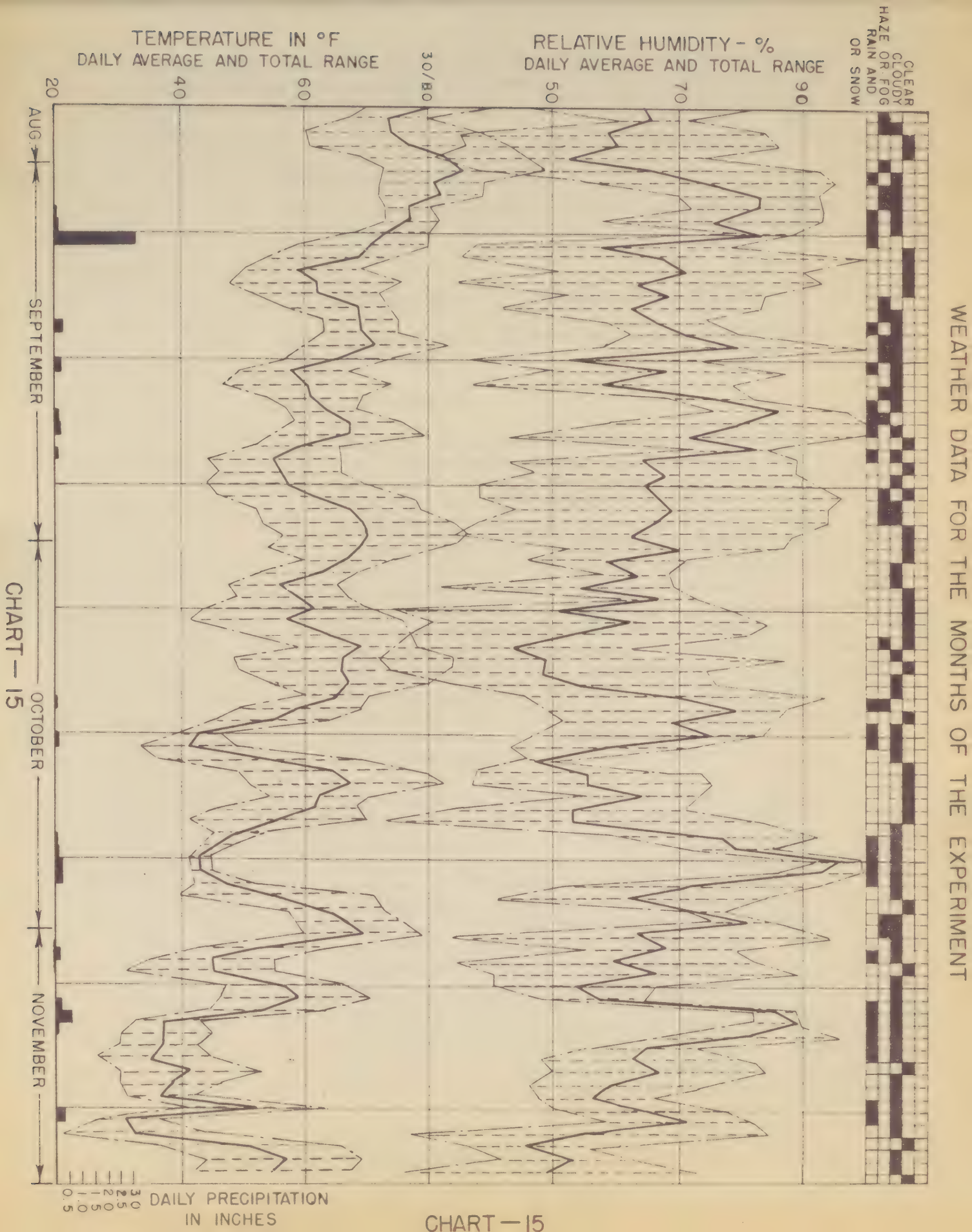
a. Subjects. One hundred (100) soldiers were selected from Company B and one hundred (100) from Company C of the Fifth Battalion, Armored Replacement Training Center. These men had recently been inducted into the army and were in their first week of basic training. Selection was on the basis of platoons rather than on an individual volunteer basis for two reasons: First, there would be less interference with training; and second, a random sample would be obtained. Men were lost from the experiment from time to time as a result of unavoidable transfer, etc. Consequently, at the end of the study 84 of the original 100 men remained in each group (168 in all). The subjects were white men from all sections of the United States, approximately one half being between 18 and 20 years of age, one quarter between 20 and 25 and one quarter between 25 and 37. This distribution agreed closely with that of the Army as a whole. The average weight for the entire group at the beginning of the study was 157 pounds and at the end of the study 160 pounds.

b. Environment. The experiment was carried out during the twelve weeks between 26 August and 13 November, 1943. August was dry and very hot. October was cooler with comparatively little rainfall. November was cold and damp. (See Chart 15)

c. Activity. A brief outline of the training program of the men during the experimental period follows:

<u>Week</u>	<u>Activity</u>
1 - 2	Orientation, School of Soldier. Chiefly indoor lectures and demonstrations.
3 - 4 - 5	Use and firing of rifle, carbine and tommy gun. Chiefly outside duty on ranges.
6	Preliminary work on Cal. 30 MG. Preparation for infiltration and village fighting courses.
7	Firing Cal. 30 MG on ranges.
8	Chiefly outside review of training.
9 - 10 - 11	Driving light and medium tanks on ranges.
12	Firing 76 mm howitzer, 76 mm tank guns and 81 mm mortar.

WEATHER DATA FOR THE MONTHS OF THE EXPERIMENT



In addition to the above, the men had 3 hours of dismounted drill and 3 hours of physical training each week; a four-hour march each week during weeks five through seven; and a twenty-five mile march during week eight.

d. Clothing, Food and Shelter.

- (1) The men wore standard fatigue uniforms consisting of coveralls, helmet liners, canvas leggings and leather shoes.
- (2) Diet consisted of regulation army garrison rations.
- (3) Men lived in a standard army barracks throughout the experiment.

e. Dosage of Atabrine.

- (1) Atabrine was administered at the noon mess by one of the officers assigned to the project. In the twelfth week when Company B was placed on full therapeutic doses, it was administered at each meal. On entering the mess hall each man picked up a cup of water, took a tablet, and after swallowing the tablet and drinking water, called his name to the officer who checked the men as they passed. A similar procedure was followed in the field. In the fourth week it was discovered that one subject had been able to expectorate the drug despite all precautions. Thereafter at intervals the oral cavities were examined after administration of the drug and as an added precaution six of the men having the lowest plasma levels were given the drug in the solution for a period of 2 weeks. No change in plasma levels of these men occurred.
- (2) The dosage and bleeding schedules for both companies for the entire experiment are given in Table 7. They were designed to cause no interference with the regular basic training program. Each group of 100 men was subdivided into two sections of fifty men each. One section from each group received 0.4 gm of atabrine a week, and the second section, 0.6 gm per week. It was originally planned to follow the two dosage regimens recommended by the Surgeon General's Office. But to meet these schedules would have necessitated keeping the men on the Post every Sunday for three months which, for reasons of morale, was felt to be inadvisable; moreover, the limitation in number of samples that could be analyzed in one day required rearrangement of schedules. To trust the men to take any dose except under direct

* SGO Circ. Letter #153, August 1943

Doses in Milligrams *

SECTIONS ONE THROUGH NINE

SUN.

MON.

TUES.

WED.

THUR.

FRI.

SAT.

Co. B. Section 1	No Drug	H ₂ & H ₂ +5 100 *	100	50	50	H ₁ 50	50
Co. B. Section 2	No Drug	100	100	H ₂ & H ₂ +5 100	No Drug	H ₁ 100	200
Co. C. Section 1	No Drug	50	H ₂ & H ₂ +5 100	100	50	50	H ₁ 50
Co. C. Section 2	No Drug	100	100	100	H ₂ & H ₂ +5 100	No Drug	H ₁ 200

SECTIONS TEN AND ELEVEN

Co. A. Section 1	50	H ₂ 100	H ₁ 50	50	50	50	50
Co. B. Section 2	100	100	100	H ₂ 100	No Drug	H ₁ 100	100
Co. C. Section 1	50	50	H ₂ 100	H ₁ 50	50	50	50
Co. C. Section 2	100	100	100	100	H ₂ 100	No Drug	H ₁ 100

SECTIONS TWELVE AND THIRTEEN

Co. B. Both Sections	500	200	100	100	100	100	100
Co. C. Both Sections	No Drug	H _x No Drug	No Drug	H _y No Drug	No Drug	No Drug	H _z No Drug

H₁ and H₂ ----- 1130 hrs.

H₂ + 5 ----- 1630 hrs.

H_{a,b,c}-----Samples During Therapeutic Dosage

H_{x,y,z}-----Samples for Disaway

observation by an officer was also considered inadvisable. Dosage schedules for the first nine weeks were therefore slightly altered from the standard SGO regimens, as indicated by Table 7. The dosage and bleeding schedules of the two sub-groups receiving 0.4 grams per week and the two sub-groups receiving 0.6 grams per week were so designed that there would be a minimum of difference in pattern of dosage between the pairs of groups receiving identical total quantities weekly.

- (3) Beginning with the 10th week the men were kept on the Post continuously for the remaining three weeks and the schedules revised (Table 7) to fit the SGO schedules.
- (4) At the beginning of week 12, Company B was put on full therapeutic doses of atabrine as described in SGO letter No. 153. Both sections of this company were given 0.5 gm atabrine on Sunday, 14 November; (0.1 gm at breakfast, 0.2 gm at lunch, and 0.2 gm at supper) 0.3 gm daily for the next five days (0.1 gm at breakfast, lunch and supper) and 0.1 gm at breakfast on the seventh day. Blood samples were taken before the noon meal on the 4th, 5th and 6th days (18, 19, and 20 November).
- (5) After the last dose for week 11, atabrine was discontinued in Company C in order to determine the rate of decline of the plasma atabrine level.

f. Observations. A brief medical history which included age, state of birth, history of suppressive or definitive malaria therapy, history of jaundice, and general statement as to previous health, was obtained on each man. A daily check was made during the study of all visits to the dispensary by experimental subjects and by the other soldiers from both companies. Records were kept of all complaints except those obviously having no possible connection with administration of atabrine; e.g. trauma and upper respiratory infections. All members of the experimental group hospitalized for any reason were followed during hospitalization and atabrine dosage was continued unless contraindicated. Blood samples were taken regularly on certain men who were hospitalized for minor injuries. Direct questioning as to possible gastroenteric tract symptoms was scrupulously avoided. For the sake of morale, however, the medical officers in charge of the study were more than ordinarily receptive to the minor complaints of the men.

g. Blood Sampling.* Blood samples (30 ml) were taken at 1130 hrs., prior to noon mess, throughout except when special samples were

* For full details concerning management of blood samples see Section I.

drawn at 1630 hrs. in order to determine peak concentrations following individual doses of atabrine. The first sample of each week was designated as H₁, the second sample H₂, and the sample taken for peak concentration H₂+5. (See Appendix D, Par. 2)

h. Presentation of Data.

- (1) Tabulation of raw data. Complete records of the plasma atabrine levels for all subjects and for all bleedings, listed by weeks and time of bleeding are tabulated by Company and Section in Appendix E. Levels obtained on therapeutic dosages and the die-away concentrations obtained during the twelfth week as well as those obtained with the suppressive dosages are included. From this complete tabulation one can study closely the behavior of each experimental subject throughout the experiment.
- (2) The geometric mean levels and standard geometric deviations for each group of subjects have been determined for every set of blood samples. These parameters were obtained both graphically from log-probability plots of the data and by direct computation and the two were in close agreement (less than + 1 micrograms). In Tables 8, 9, 10, 11 and 12, the statistical parameters for all the data are summarized, including the geometric mean (\overline{MAM}_G) and the standard error of the mean (expressed as the 68% range of the means,) and the dispersion of the individual plasma atabrine concentrations about the geometric mean (in terms of the standard geometric deviation and as the 68% range of dispersion) of the individual levels. The arithmetic mean plasma atabrine level is also given for each set of data in these Tables.

2. Results.

a. Time required to reach equilibrium. The characteristic behavior of the group mean plasma levels may be seen from inspection of Charts 16 and 17 where it will be noted that the time required to reach equilibrium level was in general the same - without regard to magnitude of dose.* Variability with respect to this secular trend was wide,

* The apparently irregular rhythmic behavior of certain groups is the result of a differing interval between dosage and sampling. The erratic results which occurred in the week of 10-15, Chart 16 and 10-22, Chart 17 were the result of aberrations in the chemical method which affected all samples on that day.

** These tables will be found at the end of this section.

MEAN₆ PLASMA ATABRINE LEVELS OF TWO GROUPS OF MEN RECEIVING 0.4 GM./WEEK

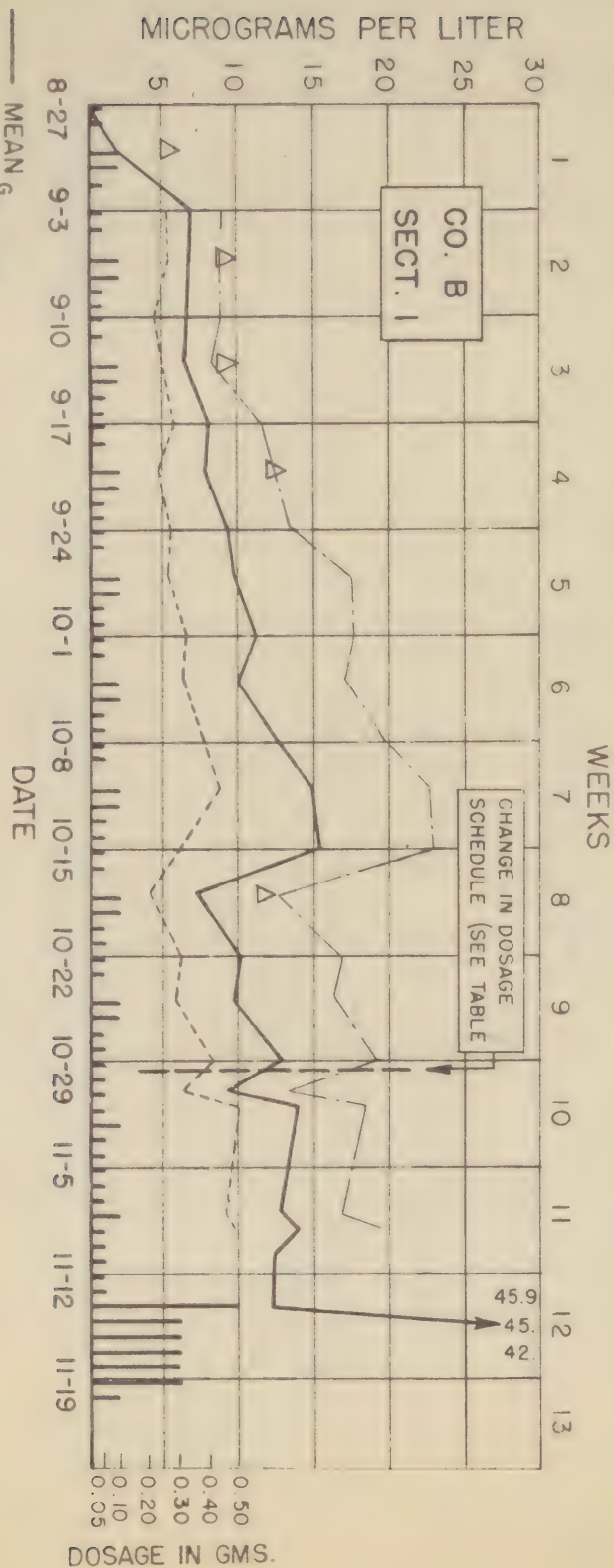
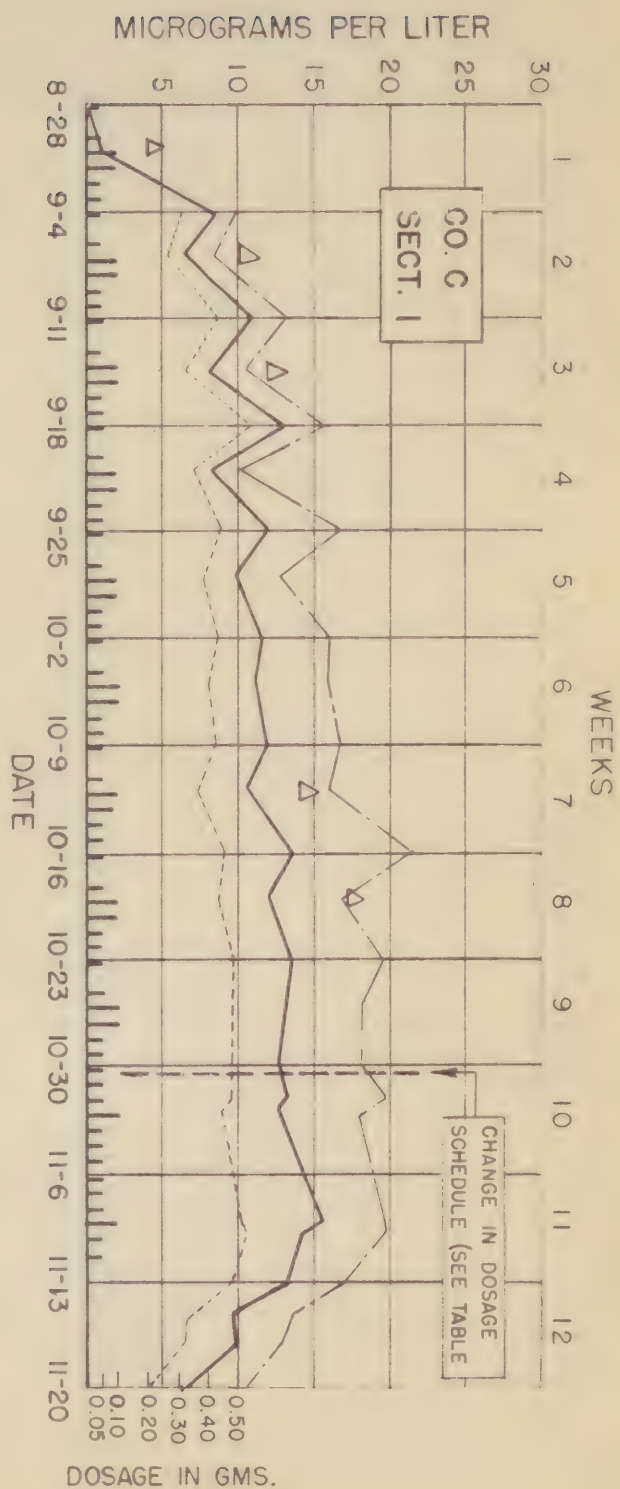


CHART - 16

MEAN_G PLASMA ATABRINE LEVELS OF TWO GROUPS OF MEN RECEIVING 0.6 GM./WEEK

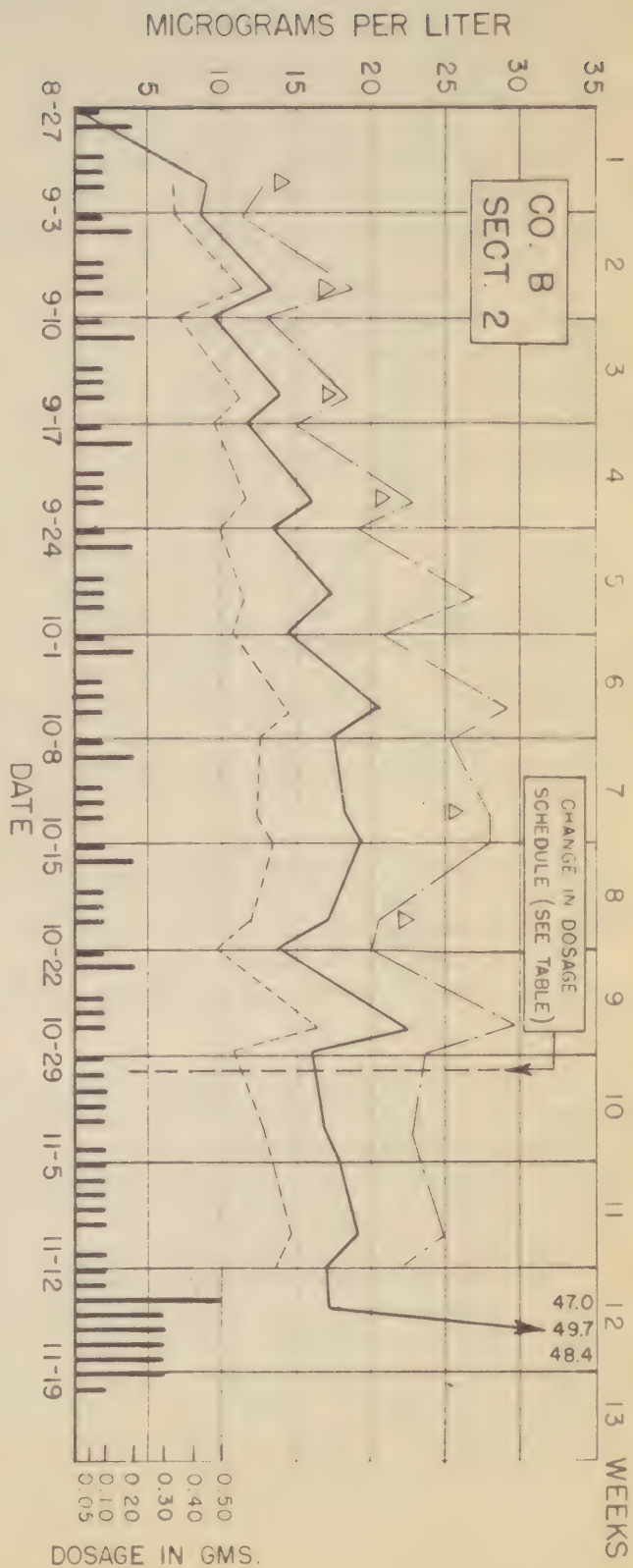


CHART -17

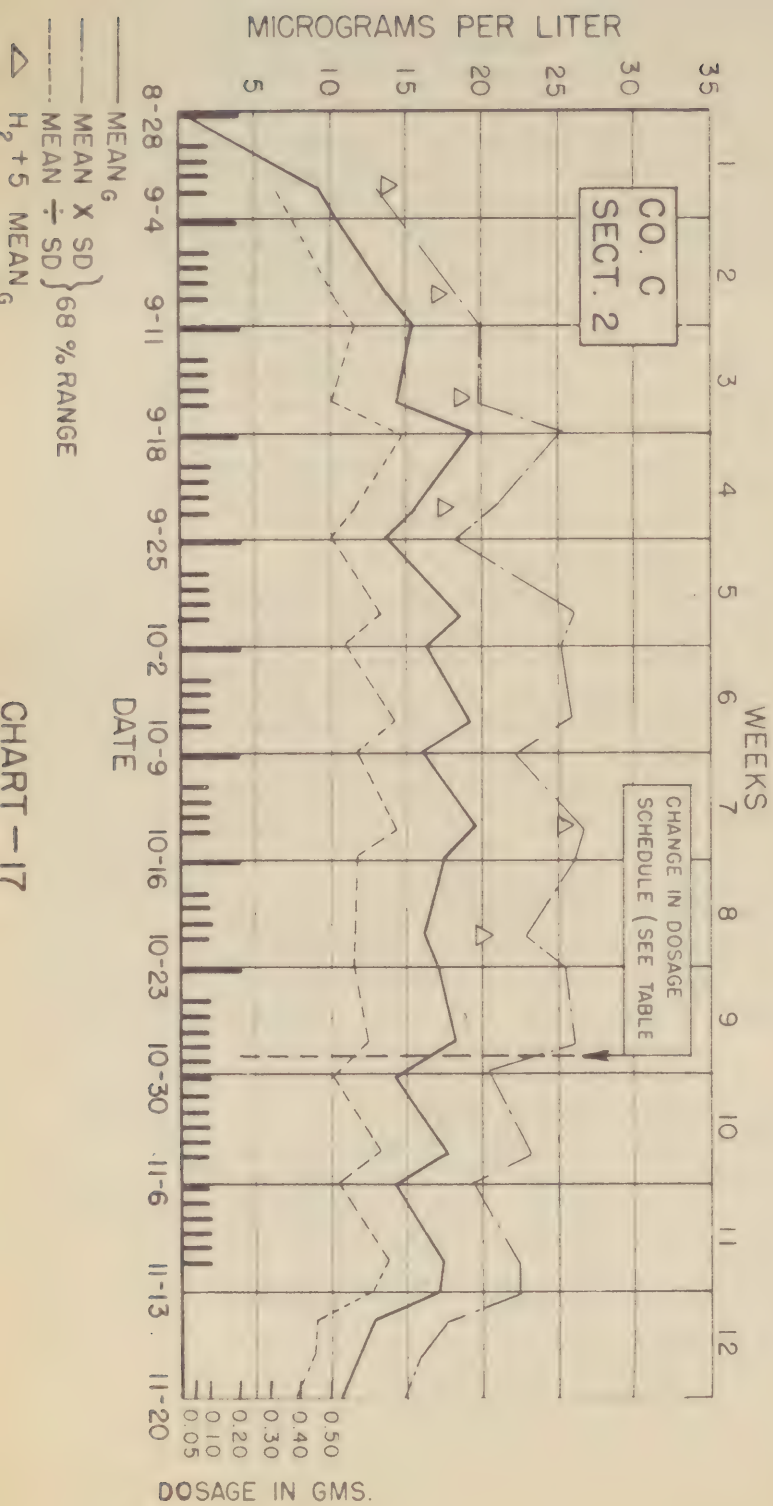


CHART -17

as will be seen later. Approximately 8%, for example, reached a level in two weeks from which they did not subsequently depart significantly. Fourteen per cent appeared to have reached equilibrium levels by the 4th week, whereas 9% may not be considered to have stabilized at all since their highest plasma levels occurred in from the 8th to 11th weeks. Then, therefore, one speaks of equilibrium stabilization with the dosage it is to be recognized that this is a term of convenience and must remain at the most a concept which is applied in this discussion to the mean plasma atabrine level of a group and not the level for an individual within the group. In keeping with the initial statement in this section, during the second and third weeks the rates of increase in plasma atabrine were quite constant; accordingly, individuals who stabilized in the earlier weeks, in general did so at levels which were lower than were those of individuals who reached their equilibrium levels in the sixth or seventh weeks. Special mention is made of the behavior of these low men at this time for two reasons: first, this portion of the group will merit special consideration in later discussion of variability in equilibrium levels and second, to point out that the time to reach equilibrium is determined not by great differences in the rate at which a given plasma level is approached, but rather that with the rate constant, it is the plasma level characteristic of the individual which determines the so-called time of equilibrium.

b. Magnitude of Group Equilibrium Level. The group mean plasma levels at equilibrium were found to be directly proportional to the dosages (Chart 18); on a regimen of 0.4 gm/wk the mean level was 12.2 micrograms per liter; with 0.6 gm/wk, a mean of 17.2 micrograms per liter was attained. These values are the mean_G of all H₁ and H₂ values of weeks 7 to 11 inclusive. Alterations in time relationship between dosage and sampling may change group values. However, when the dosage pattern was changed from that regularly followed to that recommended by the SGO, no differences in mean plasma levels were noted, c.f. Charts 16, 17, 18.

c. Variability in Individual Equilibrium Levels. The dispersion of individual values about the mean as represented by the standard deviation of the data is shown graphically in Charts 16 and 17. The values included by a range of 1 sigma on either side of the mean are also listed in the tables. From the above and the standard error of the means, the stability of the means becomes apparent.

Not evident from inspection of either the charts or tables is the wide variance of certain individuals whose plasma levels fall outside the 68% range and who are characterized by either consistent low values or by high values which resulted from sudden departure from previous levels and which then, declined somewhat, but in general, remained elevated above the remainder of the group. In view of the small size of the universe of study the validity of special consideration of the small numbers of subjects who lie at the extremes may be questioned since the sample was insufficient to make certain that one universe only was under study. One assumes, nonetheless, that with a sufficiently large sample the discontinuity would be eliminated. The characteristics of the group at the extremes are worthy of discussion, however since they illustrate types of behavior which may be of great practical importance.

CHART-18

COMPARISON OF MEAN_G WEEKLY PLASMA ATABRINE LEVELS
IN TWO GROUPS OF MEN ON DIFFERENT DOSAGE SCHEDULES
(H₁ VALUES)

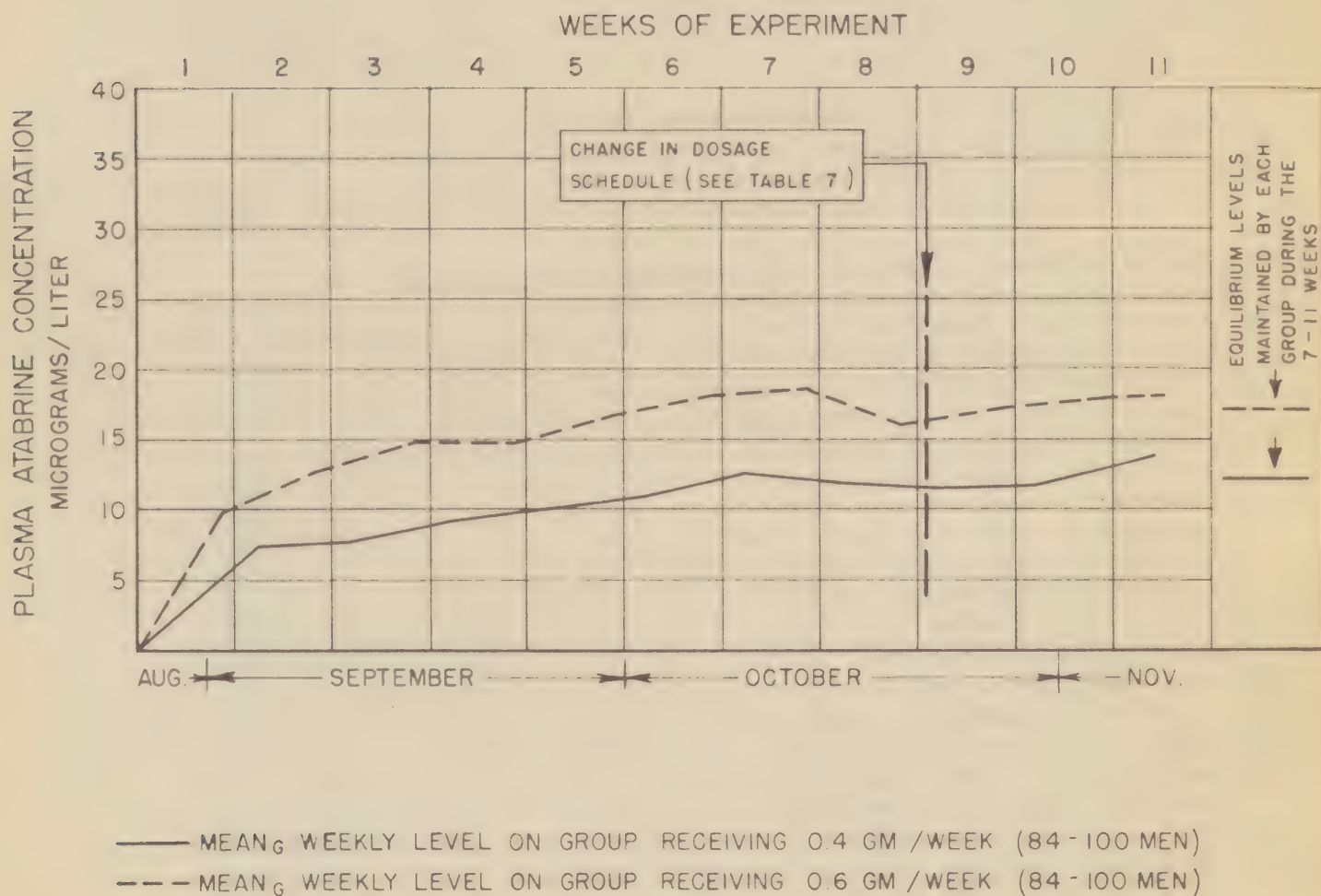


CHART-18

Approximately 7% of men showed unusually low levels as compared with the levels of the remainder of the group in both the 0.4 gm and 0.6 gm groups. As mentioned above these men reached equilibrium earlier and maintained consistent low levels, this being apparently a characteristic of individual response to the drug. That the low values were not the result of failure to take the drug or to lack of solution of the tablet in the gut was established by the administration of atabrine in solution. This resulted in no change in plasma values. Another 7% of the subjects in both the 0.4 and 0.6 gm groups exhibited the phenomenon of breakaway with more or less sudden departures from previous plasma levels to exceptionally high ones. This change to high levels occurred at various times between the 3rd to 8th week, there being no regularity in its appearance. After achieving the new high level the behavior varied. In the main the breakaway was followed by a gradual decline to a new level significantly higher than either the level from which the subject had previously departed, or the level for the group. These sporadic increases in plasma level were not associated with clinical evidence of toxicity nor was there associated evidence of liver damage. (See Appendix C, Section V)

Typical curves of plasma atabrine levels illustrating the performance of men with unusually high or low levels as compared with the usual response are presented in Chart 19. A possible explanation of these widely variant levels is given in Appendix C, Section V.

d. Significance of variability. The wide dispersion of values about the mean becomes a matter of considerable practical importance in any consideration of the extent of protection afforded a population by any standard dosage regimen. From the point of view of characterizing the population as a whole the mean and standard deviation are useful tools. From the point of view of management of troops in the field, however, it is the status of the percentiles of the group with the lowest plasma levels that most concerns us since we may properly assume, other things being equal, that this fraction of the group has the least protection and will manifest the highest malaria attack rate. With this in mind we examine the population in the different weeks with respect to the percentages of the total which exceed or fail to achieve any arbitrarily selected plasma level. In Charts 20 and 21 the distribution of concentration in each week is plotted as a single curve. These are smoothed probability curves obtained from the calculated geometric means and standard geometric deviations of the data.* They represent the most probable prediction curves which emerge from the present study. Applying the level of 10 micrograms, for example, to these charts one finds (Chart 20) that at equilibrium (weeks 7-11) 37% of the group receiving 0.4 gm/wk were still below this level whereas only 7% of the 0.6 gm/wk group (Chart 21) were below the level when stabilized. It is also apparent, even in the 0.6 gm group, that the maximum

* The curve for week 4 (Fig. 21) is incongruous and has been omitted. Data upon which it was derived are unreliable because abnormally high results were obtained from all analysis from the day of sampling.

CHART-19
DIFFERENCES IN
PLASMA ATABRINE LEVELS OF INDIVIDUALS RECEIVING 0.6 GM./WEEK

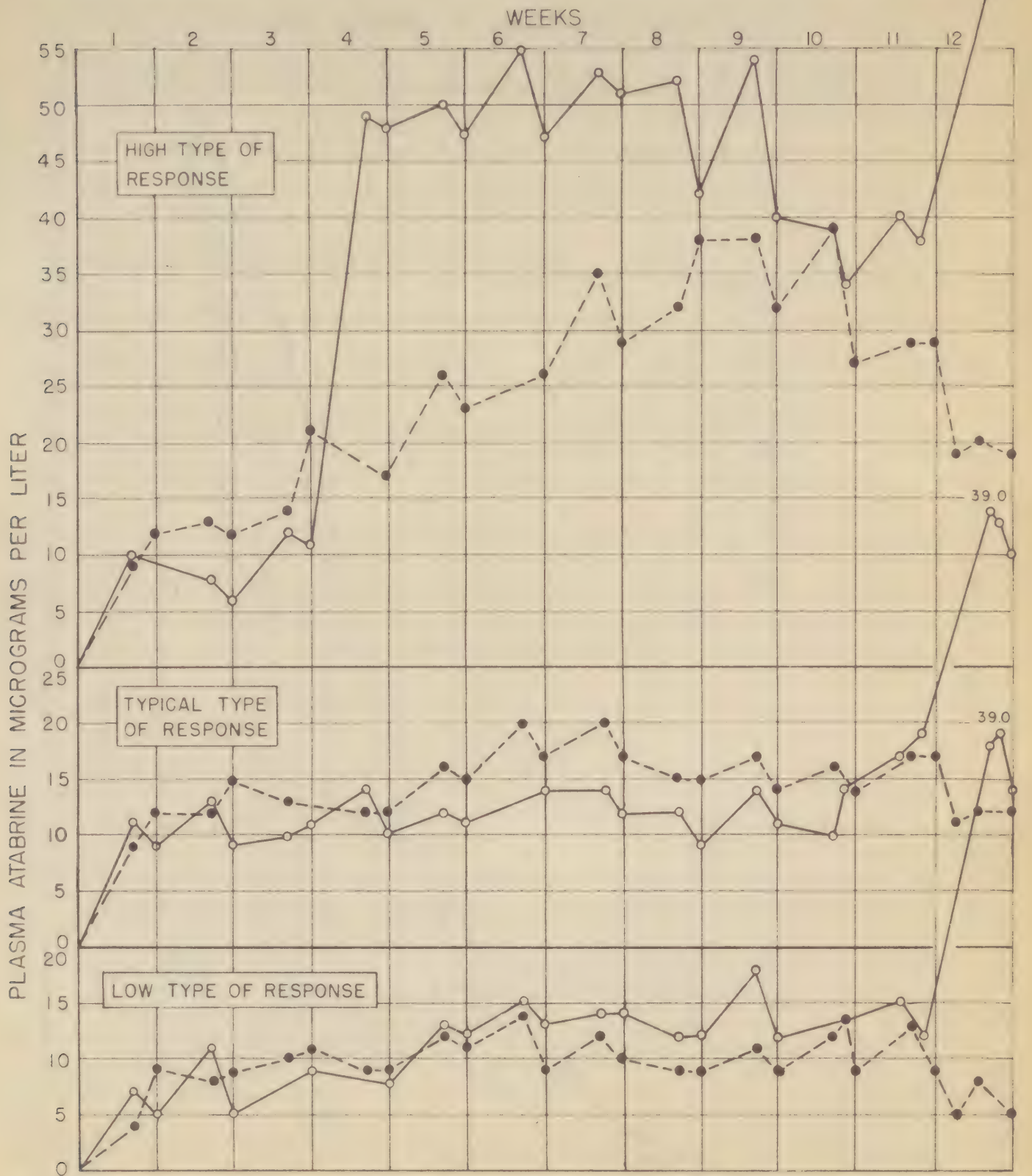


CHART-19

CUMULATIVE FREQUENCIES OF MINIMUM WEEKLY PLASMA ATABRINE LEVELS OF GROUPS OF MEN
(A.R.T.C. COMPANIES B & C) RECEIVING 0.4 GM. / WEEK

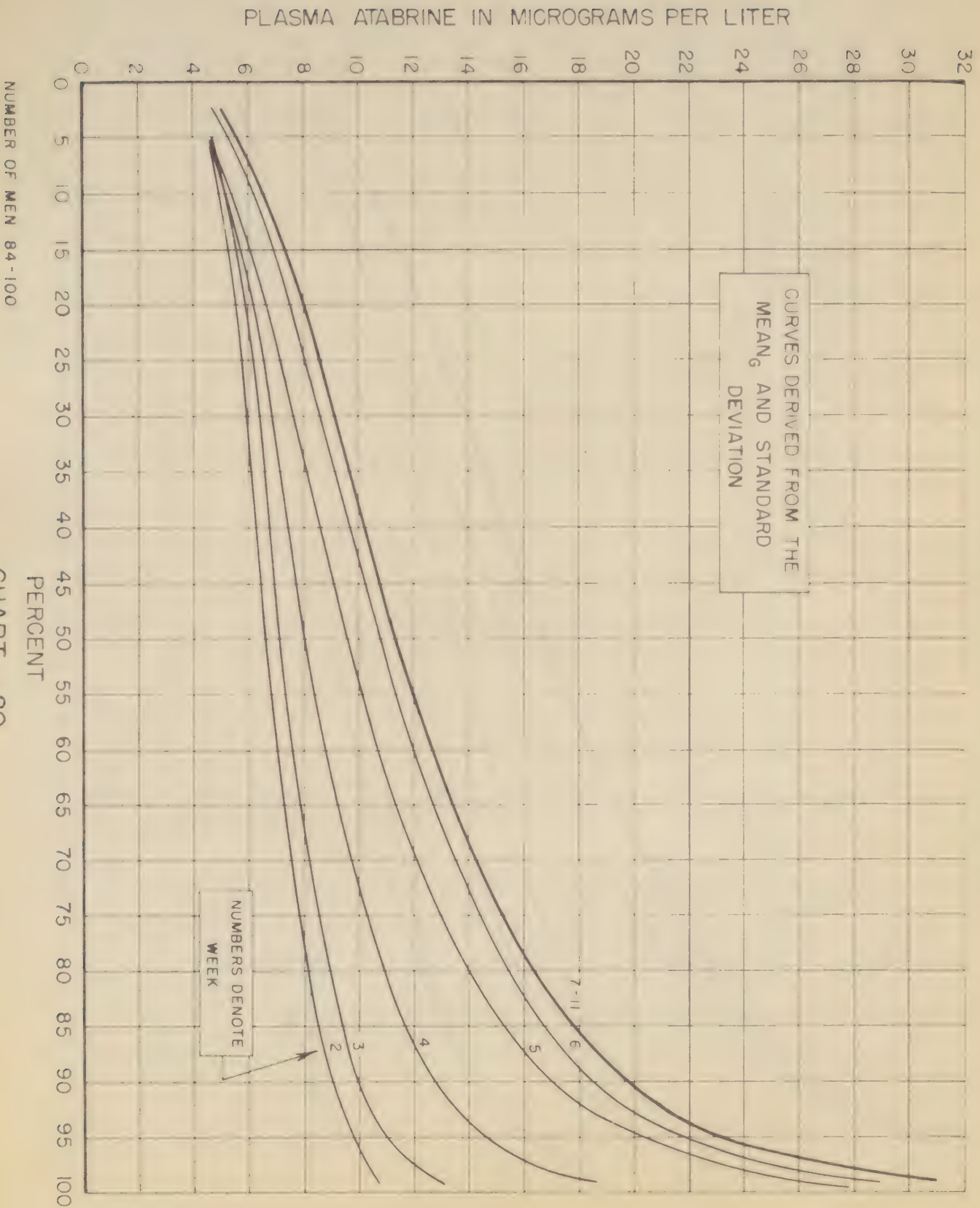


CHART - 20

CUMULATIVE FREQUENCIES OF MINIMUM WEEKLY PLASMA ATABRINE LEVELS OF GROUPS OF MEN
(A.R.T.C. COMPANIES B & C) RECEIVING 0.6 GM./WEEK

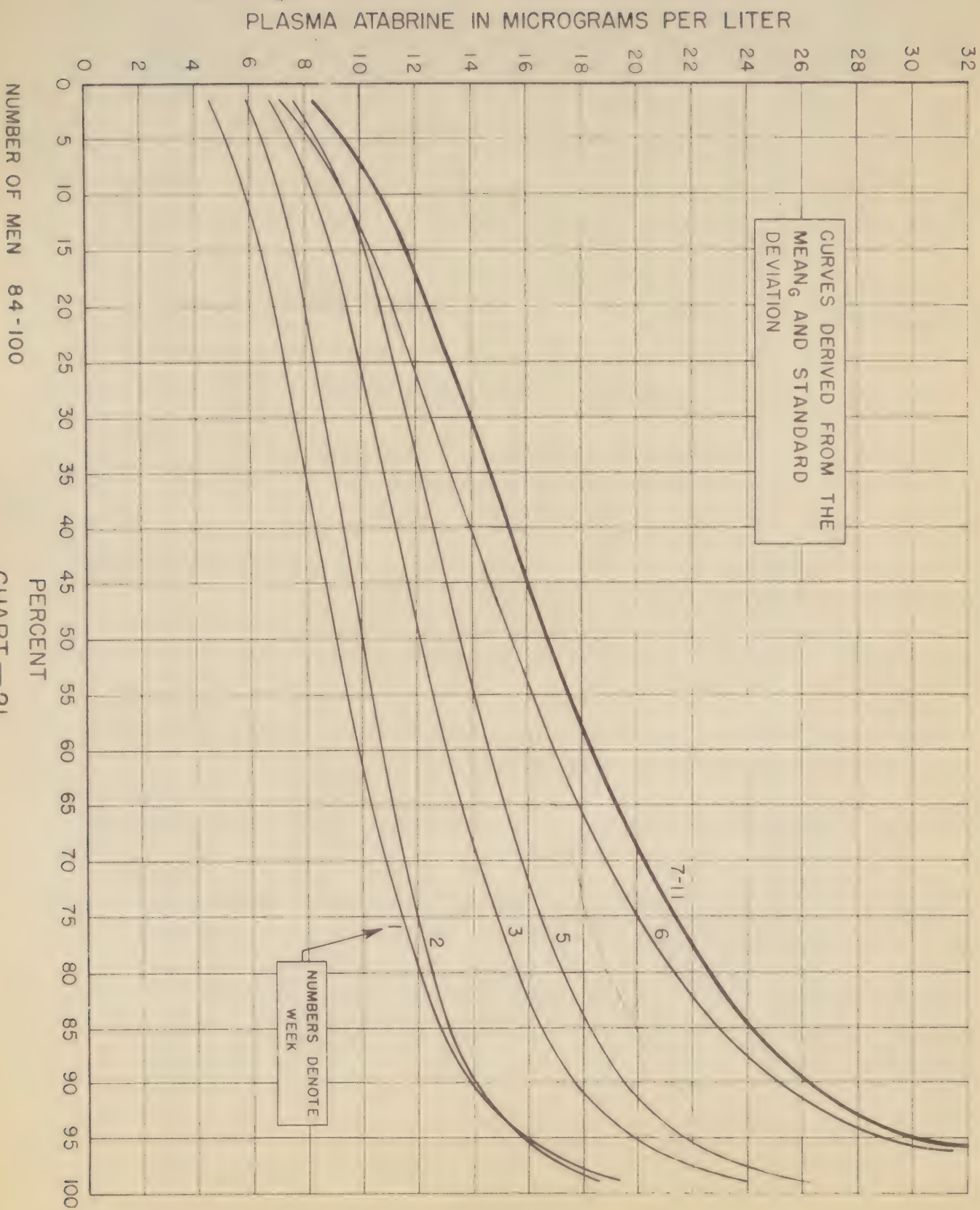


CHART-21

level for the group is not achieved until the 7th week of administration. Another significant fact which emerges from comparison of Charts 20 and 21 is that the lowest 5% of men who received 0.6 gm/wk had higher plasma levels at the end of the 2nd week than did the lowest 5% of men on 0.4 gm/wk when the latter group were at equilibrium from the 7th to 11th weeks.

BLEEDING H₁

WEEK	Date	NO. of MEN	MEAN ^G	68% RANGE of MEAN ^G		DISPERSION	MEAN ^A
Number	Date						
2	Sept 3	49	6.7	6.4-6.9	1.32	5.1-8.6	6.7
3	10	48	6.3	4.3-6.6	1.39	4.5-8.8	6.4
4	17	45	8.0	7.6-8.4	1.39	5.8-11.2	8.4
5	24	43	9.1	8.4-9.9	1.72	5.3-13.2	11.4
6	Oct 1	41	11.0	9.9-11.6	1.69	6.5-17.7	12.8
7	9	41	12.5	11.6-13.4	1.58	7.9-19.7	15.0
8	17	40	15.2	14.6-16.2	1.56	10.1-22.7	16.7
9	22	41	10.0	9.4-11.0	1.64	6.2-16.7	11.5
10	29	36	12.7	11.9-13.6	1.50	8.5-19.0	13.8
10	Nov 2	42	13.6	12.9-14.2	1.36	10.0-18.4	14.3
11	9	41	13.8	13.1-14.6	1.40	9.9-19.4	14.7

BLEEDING H₂

WEEK	Date	NO. of MEN	MEAN ^G	68% RANGE of MEAN ^G		DISPERSION	MEAN ^A
Number	Date						
1	Aug 30	50	1.6				1.6
2	Sept 6	46	6.7	6.5-7.0	1.28	5.3-8.6	6.8
3	13	47	6.3	6.1-6.6	1.31	5.0-8.3	6.5
4	20	47	7.2	7.3-8.3	1.59	4.9-12.4	7.2
5	27	44	9.5	8.7-10.4	1.78	5.3-17.1	11.9
6	Oct 4	42	10	9.6-11.1	1.65	6.3-17.0	11.3
7	11	41	11.7	13.3-15.7	1.53	8.3-22.5	16.3
8	18	40	7.2	6.6-7.9	1.73	4.2-12.5	9.2
9	25	42	9.6	8.9-13.1	1.68	5.7-16.2	11.2
10	Nov 1	41	9.1	8.6-9.6	1.45	6.3-13.1	9.8
11	8	42	12.4	11.9-13.0	1.34	9.2-16.7	13.0

BLEEDING H₂ + C

WEEK	Date	NO. of MEN	MEAN ^G	68% RANGE of MEAN ^G		DISPERSION	MEAN ^A
Number	Date						
1	Aug 30	50	5.2	5.0-5.4	1.32	4.0-6.8	5.5
2	Sept 6	49	7.6	7.4-7.9	1.29	5.9-9.8	7.7
3	13	44	9.0	8.6-9.7	1.57	5.8-14.2	9.1
4	20	44	12.4	11.7-13.1	1.49	8.3-18.4	13.6
5	Oct 8	38	11.8	10.9-12.8	1.63	7.3-19.3	13.5

MEAN_G AND CHARACTERISTICS OF WEEKLY PLASMA ATAPIRIN LEVELS

FOR

COMPANY B SECTION 1

0.4 gm/wk

BLEEDING H₁

WEEK	68%			DISPERSION			
Number	Date	NO. of MEN	MEAN ^G	RANGE of MEAN ^G	1σ	RANGE	MEAN ^A
2	Sept 4	45	8.5	8.4-8.7	1.16	7.4-9.9	8.6
3	11	43	10.7	10.4-10.9	1.24	8.6-13.3	10.9
4	18	44	13.1	12.8-13.2	1.20	10.9-16.5	13.3
5	25	41	12.1	11.7-12.7	1.36	8.9-16.5	12.7
6	Oct 2	43	11.6	11.0-12.1	1.36	8.6-15.8	12.3
7	9	40	12.0	11.4-12.7	1.41	8.5-16.9	12.9
8	16	43	13.9	13.0-14.9	1.55	9.0-21.6	15.4
9	23	42	13.5	12.8-14.2	1.41	9.6-19.0	14.4
10	30 NOV	39	12.7	12.0-13.4	1.41	9.0-17.8	13.6
11	3	42	12.7	12.1-13.4	1.42	8.9-18.0	13.6
12	10	42	14.3	13.6-15.0	1.36	10.5-19.4	15.0

BLEEDING H₂

WEEK	NO. of MEN	MEAN ^G	68% RANGE of MEAN ^G	DISPERSION	MEAN ^A
Number	Date			1σ	
1	Aug 31	4.2			1.0
2	Sept 7	4.5	6.7	1.26	6.9
3	14	4.7	6.3	1.27	8.5
4	21	4.1	8.5	1.19	8.7
5	28	4.3	9.8	1.29	10.5
6	Oct 5	4.2	11.2	1.41	12.1
7	12	4.0	10.6	1.50	11.8
8	19	4.2	12.0	1.40	14.3
9	26 Nov	3.9	13.1	1.38	13.8
10	2	4.2	13.2	1.46	14.2
11	9	4.0	13.8	1.38	14.6

BLEEDING H₂ + 5

WEEK		NO. of MEN	MEAN ^G	68% RANGE of MEAN ^G	DISPERSION		MEAN ^A
Number	Date				1σ	68% RANGE	
1	Aug 31	47	4.6	4.4- 4.8	1.39	3.3- 6.4	4.9
2	Sept 7	46	10.7	10.4-11.1	1.23	8.7-13.2	10.8
3	14	46	12.3	11.9-12.7	1.24	9.9-15.2	12.6
4	21	43	11.1	10.7-11.5	1.24	8.9-13.8	11.4
7	Oct 12	37	14.4	13.5-15.2	1.48	9.7-21.3	16.5
8	19	41	17.9	17.1-18.8	1.34	13.4-24.0	18.7

MEAN^G AND CHARACTERISTICS OF
WEEKLY PLASMA ATABRINE LEVELS

FOR

COMPANY C SECTION 1

0.4 gm/wk

BLEEDING H₁

WEEK	Number	Date	No. of MEN	MEAN _G	68% RANGE of MEAN _G	DISPERSION		MEAN _A
						68	68% RANGE	
2	Sept 3		47	8.9	8.6-9.3	1.28	6.9-11.4	8.8
3	10		45	9.6	9.2-10.0	1.43	7.1-12.9	9.8
4	17		42	11.8	11.4-12.2	1.25	9.5-14.7	11.6
5	24		41	13.6	12.9-14.3	1.37	9.9-18.6	15.1
6	Oct 1		45	14.7	14.0-15.5	1.39	10.6-20.5	15.9
7	8		41	17.5	16.5-18.4	1.41	12.3-24.7	18.7
8	15		40	19.3	18.2-20.6	1.46	13.2-28.3	18.8
9	22		40	13.6	12.8-14.4	1.46	9.3-19.8	14.7
10	29		42	16.0	15.0-17.0	1.48	10.8-23.7	17.0
11	Nov 5		41	17.9	17.2-18.7	1.31	13.7-23.6	19.9
12	12		40	17.5	16.9-18.2	1.28	13.7-22.3	18.1

BLEEDING H₂

WEEK	Number	Date	No. of MEN	MEAN _G	68% RANGE of MEAN _G	DISPERSION		MEAN _A
						68	68% RANGE	
1	Sept 1		49	9.2	8.8-9.7	1.37	6.7-12.7	10.0
2	8		45	13.9	13.3-14.3	1.25	11.1-17.7	13.4
3	15		43	14.2	13.6-14.8	1.30	10.9-18.4	14.0
4	22		43	16.2	15.4-17.1	1.41	11.5-22.8	16.0
5	29		44	17.5	16.4-18.8	1.56	11.2-26.8	18.8
6	Oct 6		43	20.4	19.3-23.1	1.42	14.3-29.0	21.8
7	13		44	18.3	17.2-19.5	1.58	12.1-27.7	21.5
8	20		42	17.3	16.3-18.4	1.48	11.7-20.3	16.7
9	27		43	22.4	21.4-23.3	1.32	16.1-29.5	23.5
10	Nov 3		41	16.9	16.2-16.7	1.35	12.6-22.8	17.7
11	10		41	19.0	18.1-19.7	1.30	14.5-24.6	19.6

BLEEDING H₂

WEEK	Number	Date	No. of MEN	MEAN _G	68% RANGE of MEAN _G	DISPERSION		MEAN _A
						68	68% RANGE	
1	Sept 1		48	14.1	13.6-14.7	1.33	8.5-18.8	14.0
2	8		45	16.9	16.3-17.6	1.32	12.8-22.5	17.0
3	15		44	17.3	16.6-18.0	1.29	13.3-22.4	18.1
4	22		43	20.8	20.1-21.8	1.33	15.7-27.7	20.5
7	Oct 13		44	25.9	24.6-27.2	1.39	18.7-35.7	28.1
8	20		41	22.1	21.0-23.2	1.37	16.1-30.3	21.4

MEAN_G AND CHARACTERISTICS OF
WEEKLY PLASMA ATABRINE LEVELS

FOR

COMPANY B SECTION 2

0.6 gm/wk

BLEEDING H₁

WEEK	Number	Date	No. of MEN	MEAN ^o	68% RANGE of MEAN ^o	DISPERSION		MEAN ^A
						Std	68% RANGE	
2	Sept 4		52	11.2	10.9-11.5	1.35	9.0-13.9	12.0
3	11		51	15.1	14.5-15.6	1.35	11.5-19.7	15.6
4	18		48	19.1	18.4-20.0	1.31	14.6-25.0	19.8
5	25		45	13.6	13.0-14.2	1.35	10.0-18.3	14.3
6	Oct 2		46	16.3	15.3-16.8	1.54	10.6-25.1	17.6
7	9		46	16.0	15.3-16.8	1.37	11.7-21.9	16.8
8	16		42	17.5	16.4-18.6	1.49	11.7-26.1	18.8
9	23		44	17.0	16.0-18.0	1.49	11.4-25.3	18.5
10	30		44	14.4	13.6-15.2	1.43	10.1-20.5	15.3
11	Nov 6		44	14.3	13.6-14.9	1.35	10.6-19.2	15.0
12	13		44	17.0	16.3-17.8	1.35	12.8-22.5	17.6

BLEEDING H₂

WEEK	Number	Date	No. of MEN	MEAN ^o	68% RANGE of MEAN ^o	DISPERSION		MEAN ^A
						Std	68% RANGE	
1	Sept 2		53	9.1	8.7-9.6	1.44	6.4-13.1	9.6
2	9		50	13.7	13.1-14.3	1.34	10.2-18.4	14.2
3	16		45	14.4	13.8-15.1	1.38	10.0-19.8	15.2
4	23		47	15.4	14.7-16.1	1.34	11.5-20.6	16.2
5	30		46	18.6	18.5-19.7	1.40	13.3-26.0	19.3
6	Oct 7		46	19.3	18.7-20.5	1.34	14.1-25.9	20.2
7	14		47	19.6	18.7-20.5	1.36	14.4-26.7	23.0
8	21		44	16.0	15.2-16.9	1.42	11.3-22.7	17.0
9	28		45	17.8	16.9-18.9	1.46	12.3-26.0	19.1
10	Nov 4		44	17.6	16.9-18.4	1.32	13.4-23.3	18.5
11	11		45	17.4	16.8-18.1	1.28	13.7-22.2	17.9

BLEEDING H₂ + 5

WEEK	Number	Date	No. of MEN	MEAN ^o	68% RANGE of MEAN ^o	DISPERSION		MEAN ^A
						Std	68% RANGE	
1	Sept 2		52	13.8	13.2-14.4	1.37	10.0-18.9	12.0
2	9		51	17.2	16.5-18.0	1.35	12.8-23.3	16.1
3	16		46	18.6	17.8-19.5	1.36	13.7-25.3	19.6
4	23		47	17.5	16.6-18.3	1.42	12.3-24.9	18.6
7	Oct 14		45	25.5	24.4-26.7	1.36	18.8-34.8	26.8
8	21		44	20.0	19.0-21.0	1.36	14.4-27.7	21.1

MEAN AND CHARACTERISTICS OF

WEEKLY PLASMA ATABRINE LEVELS

FOR

COMPANY C SECTION 2

0.6 gm/wk

WEEK	Number	Date	No. of MEN	MEAN	68% RANGE of MEAN	S.D.	DISPERSION		MEAN
							68% RANGE		
2	Sept 3 & 4		94	7.5	7.3- 7.7	1.29	5.8- 9.7	7.8	
3	10&11		96	8.2	7.9- 8.5	1.47	5.6-12.0	8.8	
4	17&18		89	10.2	9.9-10.6	1.43	7.1-14.7	10.9	
5	24&25		84	10.4	9.9-11.0	1.59	6.6-16.7	12.1	
6	Oct 1 & 2		84	11.0	10.6-11.5	1.52	7.3-16.7	12.5	
7	8 & 9		81	12.5	11.7-13.1	1.50	8.3-18.8	13.9	
8	15&16		83	14.5	13.8-15.2	1.53	9.5-22.2	15.5	
9	22&23		83	11.7	11.2-12.3	1.56	7.5-18.4	13.0	
10	29&30		75	12.7	12.2-13.2	1.45	8.7-18.4	13.7	
10	Nov 2 & 3		84	13.1	12.6-13.6	1.40	9.4-18.3	13.9	
11	9 & 10		83	14.0	13.5-14.5	1.38	10.2-19.3	14.7	

WEEK		No. of MEN	MEAN	68% RANGE of MEAN	S.D.	DISPERSION		MEAN
Number	Date					68% RANGE		
2	Sept 3 & 4	99	10.0	9.7-10.4	1.30	7.7-13.1	10.6	
3	10&11	96	12.2	11.8-12.6	1.34	9.1-16.3	12.8	
4	17&18	90	15.3	14.9-15.7	1.30	11.8-19.5	15.9	
5	24&25	96	13.6	13.2-14.0	1.33	10.2-18.0	14.7	
6	Oct 1 & 2	91	15.5	14.9-16.1	1.48	10.5-22.8	16.7	
7	8 & 9	87	16.7	16.1-17.3	1.43	11.7-23.9	17.7	
8	15&16	82	18.4	17.6-19.2	1.48	12.4-27.3	20.0	
9	22&23	84	15.3	14.6-16.0	1.50	10.2-22.9	16.6	
10	29&30	86	15.1	14.5-15.8	1.46	10.3-22.1	15.6	
11	Nov 5 & 6	84	16.5	15.9-17.0	1.35	12.2-22.2	17.5	

BLEEDING H₂

2	Sept 6 & 7		91	6.7	6.6-6.9	1.27	5.3-8.5		6.9
3	13 & 14		94	7.2	7.1-7.4	1.29	5.6-9.3		7.5
4	20 & 21		88	8.1	7.8-8.4	1.43	5.7-11.7		9.0
5	27 & 28		87	9.6	9.2-10.1	1.58	6.1-15.2		11.2
6	Oct 4 & 5		84	10.8	10.2-11.3	1.54	7.0-16.5		11.9
7	11 & 12		81	12.5	11.8-13.3	1.56	8.0-19.6		14.1
8	18 & 19		82	9.4	9.0-9.8	1.53	6.1-14.4		11.8
9	25 & 26		81	11.2	10.6-11.8	1.58	7.1-17.7		12.4
10	Nov 2 & 3		83	11.0	10.5-11.5	1.52	7.2-16.7		11.7
11	8 & 9		82	13.0	12.6-13.5	1.37	9.5-17.8		13.8

BLEEDING H₂

1	Sept 1 & 2		102	9.1	8.8-9.4	1.41	6.5-12.8		9.8
2	8 & 9		95	13.7	13.2-14.3	1.30	10.6-17.8		14.1
3	15&16		88	14.3	13.9-14.8	1.34	10.7-19.2		14.9
4	22&23		90	15.8	15.3-16.3	1.37	11.5-21.6		16.7
5	29&30		90	18.1	17.3-18.8	1.49	12.5-26.8		19.3
6	Oct 6 & 7		89	19.8	19.1-20.5	1.39	14.3-27.4		21.0
7	13&14		91	18.9	18.2-19.7	1.44	13.1-27.3		22.2
8	20&21		86	16.6	16.0-17.3	1.45	11.4-24.1		17.9
9	27&28		80	19.9	19.2-20.7	1.42	14.0-28.3		19.3
10	Nov 3 & 4		85	17.3	16.6-17.8	1.33	13.0-23.0		18.1
11	10&11		86	18.1	17.6-18.6	1.29	14.0-23.4		19.7

GROUPS RECEIVING 0.40 GRAMS/WEEK

GROUPS RECEIVING 0.60 GRAMS/WEEK

MEAN AND CHARACTERISTICS OF WEEKLY PLASMA ATABRINE LEVELS

TABLED DATA FOR COMBINED ARTC GROUPS - SECTIONS 1 AND 2 OF COMPANIES B AND C

APPENDIX C

SECTION III - Effects of a Simulated Jungle Climate Upon Plasma Atebrine Levels.

1. Procedure.

a. Subjects.

- (1) Thirty (30) enlisted volunteers served as experimental subjects. Fifteen (15) were 19 years old, nine (9) 20 years, two (2) 21 years and one each 22, 24, 31 years. They came originally from all parts of the United States and had been in the Army 4 or 5 months. Their military service included reception center, basic and battle training. The men were of representative body types, and in varying states of physical fitness. After three weeks of preliminary training and testing they were divided into two groups of 15 men each. These groups, hereafter called A and B, were similar in respect to age, bodily configuration, weight, section of the country from which the men came, physical fitness, cardiovascular and thermal response to a standard work procedure. This similarity existed for both the group averages and the distributions within the groups (e.g. equal numbers of small and tall men in each group).
- (2) Throughout the study both groups were handled in exactly the same manner in all respects except in climatic exposure. One group initially resided in the simulated jungle climate, the other in the Fort Knox climate.

b. Environment.

- (1) In the hot room a hot humid (jungle) climate was simulated. During the day (0800 to 1700 hours) the dry bulb temperature averaged 90°F with extremes of 89°F to 93.5°F. The relative humidity was usually 92% to 95% with extremes of 100% and 88%. During the night (1800 hours to 0630 hours) the dry bulb temperature was 78°F to 87°F (usually 84°F) and the relative humidity 75% to 85%. It required one hour to change from day to night climate. No radiant heat was supplied. Air movement was turbulent but not greater than that made by the moving men. The men lived continuously in this climate except for a 10 minute clean-up in the morning and evening.

- (2) The outside environment varied from a hot dry summer period to cool fall weather. This climate is indicated in Chart 22, showing dry bulb temperature ($^{\circ}\text{F}$) and relative humidity as the average values for all of the readings at 15 minute intervals during each work period throughout the week. The hot humid climate is similarly plotted on the same chart.

c. Activity and Work.

Both groups followed a standard daily work procedure consisting of walking five "work-periods" a day at 2-1/2 miles in 47 minutes (approximately standard army pace) carrying a 20 pound pack. Between successive work periods there were 13 minutes of rest during which observations were made on the men. Two successive work periods in the morning and three in the afternoon (a total walk of 12-1/2 miles) constituted a standard work-day. Five work periods daily on the first five days of the week, three periods on Saturday and none on Sunday constituted the standard work-week. One hour periods of organized athletics, calisthenics or close order drill were at times substituted for work periods in the outside group. The energy expenditure during this substituted activity was comparable to that of a work period as judged by the responses of the heart rate, rectal temperature and sweating rate.

d. Clothing, Food, Water, Sleep.

During the work periods all men wore the regulation fatigue coverall, cotton shorts, socks and shoes. The food consisted of standard garrison rations and was salted to individual taste. Extra salt was not added. During work, water salted to a final concentration of 0.1% was permitted as desired. This was the only water permitted in the hot room at any time. The group outside drank salted water during the work periods, fresh water at other times. Approximately 8-9 hours were permitted for sleep.

e. Dose of Atabrine.

Both groups began taking atabrine on 9 August 1943; the same dosage and procedure being followed in each group. During six days of the first week each man received 0.1 gm with his morning and evening meals, to give a total of 1.2 gms/wk. During the following 11 weeks each man received 0.6 gm/wk, 0.1 gm daily for six days with the noon meal Monday through Friday, with breakfast on Sat-

CLIMATIC CONDITIONS DURING WORK HOURS

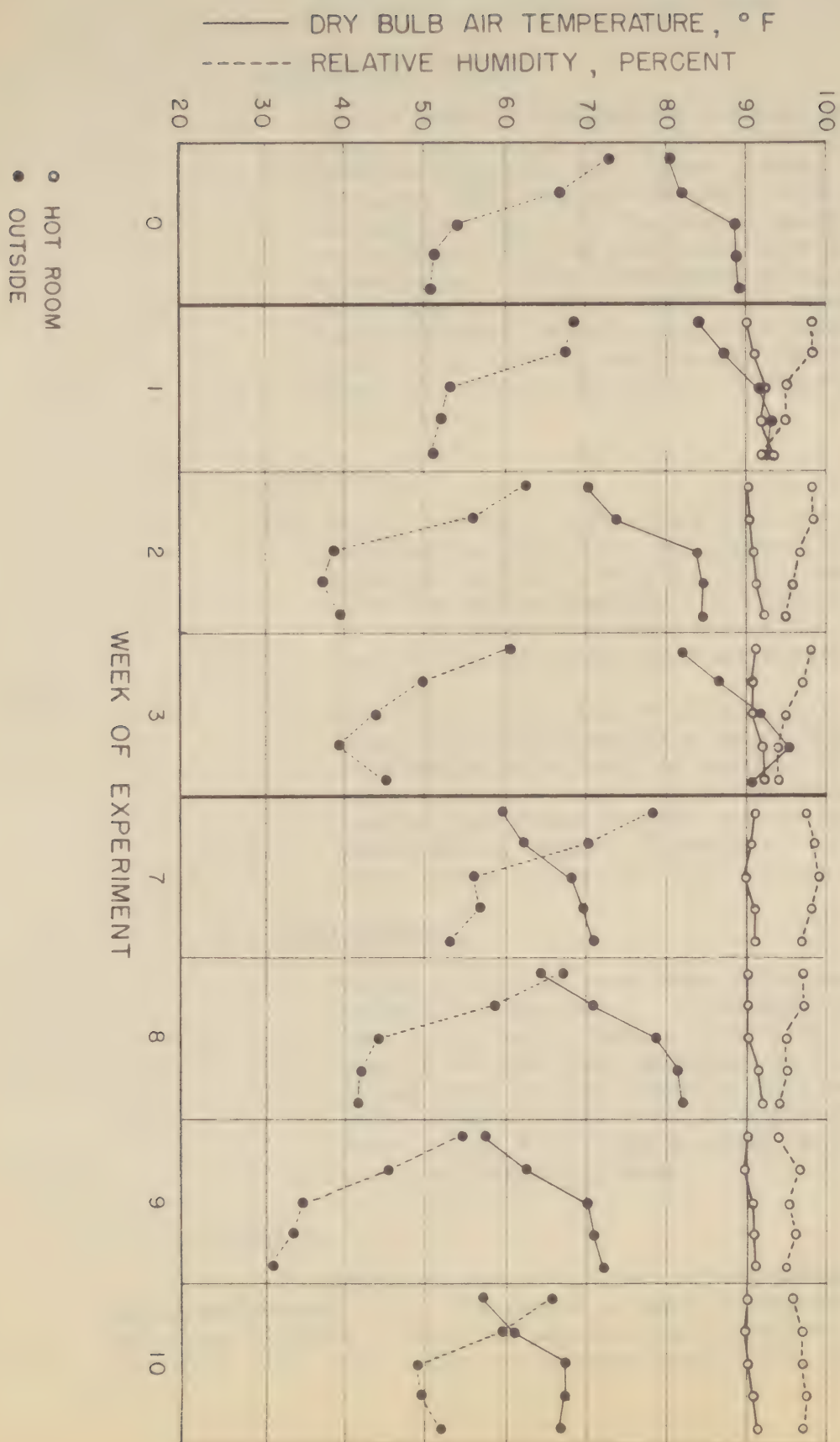


CHART - 22

urday. No drug was given on Sunday. Rigid precautions were taken to insure the swallowing of the tablets.

f. Rotation of Exposure.

After 2 weeks of preliminary training for all subjects group A entered the jungle climate while group B remained outside in the summer climate. On the same day (9 August 1943) both groups began suppressive atabrine therapy (See Par. e). The men in group A were permitted to leave the hot room from 1300 hours Saturday until 2200 hours Sunday. After seven weeks of exposure in the hot room group A was moved outside and group B entered the jungle climate. For the next four weeks group B remained continuously in this environment; the B men were not permitted to leave on Saturday afternoon and Sunday as had group A.

g. Observations.

- (1) Before and after each work period the following observations were made: (a) general appearance and symptoms, (b) heart rate and blood pressure in both erect and supine positions, (c) rectal temperature. The water intake and urine output and the weight (sweat) loss, within 10 grams, were determined.
- (2) The 24 hour water intake of the jungle group was measured daily and the 24 hour urine output and specific gravity of 10 men of each group (A and B). The urinary chloride excretion per 24 hours and the plasma chloride concentration, the plasma protein and the hemoglobin were determined at regular intervals on 10 subjects in each group before and during their exposure to heat.

h. Blood Sampling.

Thirty (30) ml of blood were withdrawn for each plasma atabrine determination. Three samples were taken each week (a) H₁ - 1115 Monday, before the first atabrine dose of the week, approximately 54 hours after the preceding dose; (b) H₂ - 0630 Saturday before the last atabrine dose of the week, 18 hours after the preceding dose; (c) H₂ + 5 - 1130 Saturday, 5 hours after the last atabrine dose of the week.

2. Results.

a. The work performance and rate of acclimatization in the hot jungle environment of the two groups of men are compared in Chart 23. The two groups differed in that the men in group A received atabrine regularly from their first day in the heat whereas group B had already received the

CHART - 23

CHANGES IN HEART RATE, RECTAL TEMPERATURE AND BLOOD PRESSURE DURING ACCLIMATIZATION TO WORK IN JUNGLE HEAT

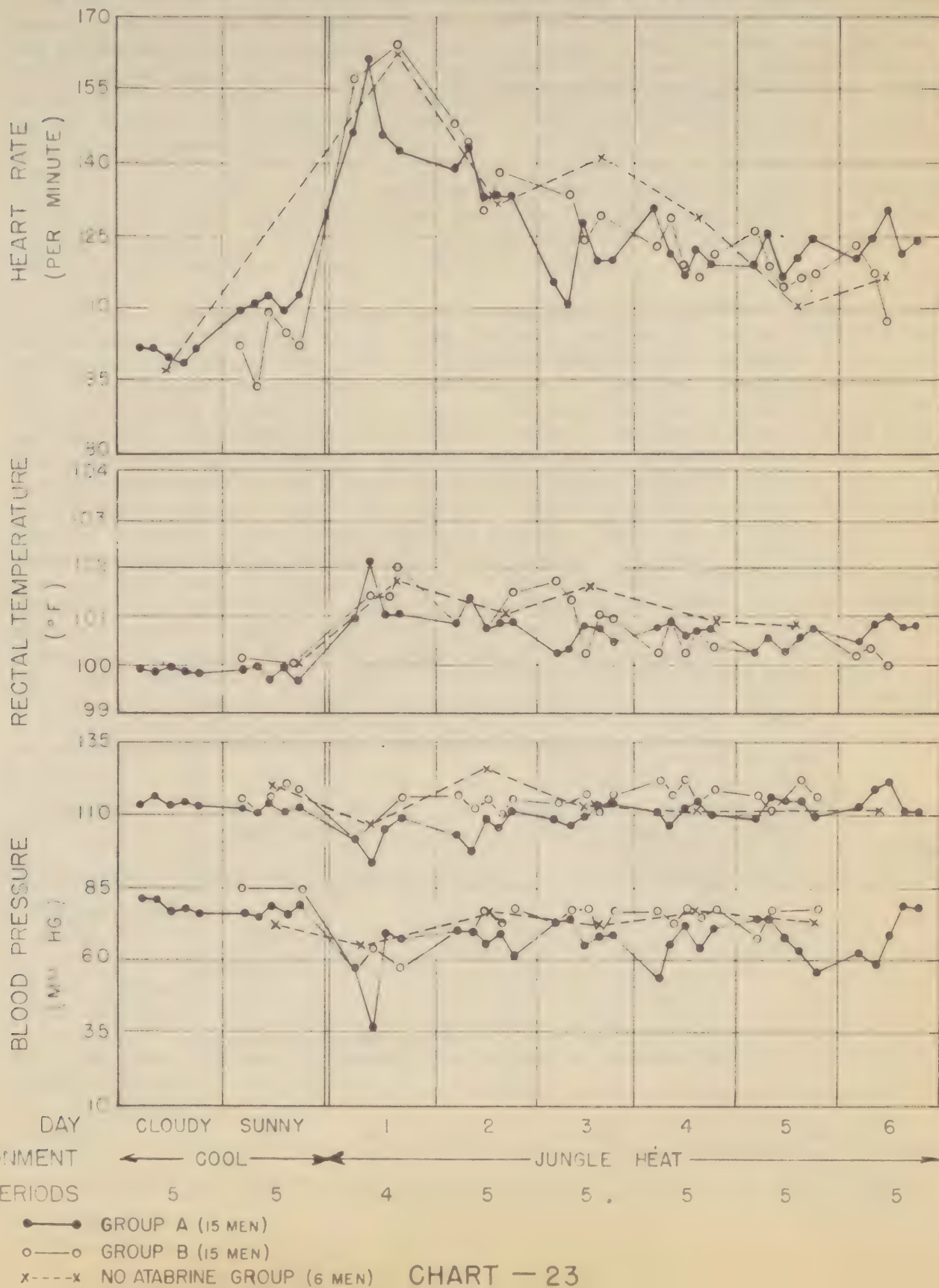


CHART - 23

drug for seven weeks before exposure to the jungle environment. For further comparison, the performance of a third group who received no atabrine is also shown on the Chart. Two important characteristics of the experiences of these three groups must be mentioned. First, the work performances on the first day and thereafter and the progress of acclimatization among the subjects did not differ in any significant way from that observed in previously reported studies of the effect of moist heat upon the performance of young men.* Second, there was no essential difference with respect to work performance or rate of acclimatization between the three groups. It is evident, therefore, that the progressive accumulation of atabrine in the body concurrent with heat exposure, or continuing to take atabrine after an equilibrium level has been established in the cool do not in any way affect the physiological reactions of young men exposed to moist heat. With or without the drug the rate of acclimatization and ability to work in a jungle climate are the same.

b. The plasma atabrine levels attained by groups A and B, who received the drug according to the same dosage schedules but under different environmental conditions are given in Tables 13 and 14** and compared in Chart 24. No difference in the rate of increase or of the final equilibrium levels will be observed between the two groups, nor was there any significant change in the equilibrium level in either groups when the subjects were shifted from one environment to the other. In this connection it should be noted that the outside exposure of group A, following their jungle exposure represented a much greater climatic shift than was experienced by group B who received their outside exposure first, during the late summer. Thus, the fact that group A showed no measurable alteration in plasma atabrine level is more significant than the fact that the level for group B did not change. The increased sweating rate experienced in the jungle environment had no effect upon the plasma atabrine level as shown in Chart 25. The equilibrium levels reached (7th through 11th weeks) by the two groups and the levels reached by the larger ARTC group who received the same weekly maintenance dosage did not differ significantly from the level of 18 micrograms/L predicted by the dosage schedule, the deviations being approximately equivalent to one standard error.

Exposure	No. Results	No. Men	Equilibrium atabrine level on 0.6 gm dosage per week Mean _G and dispersion of levels (7th through 11th weeks)		
			Mean _G	S. E. of Mean _G	Std. Geo. Deviation
Outside, ARTC group	858	85	17.2	1.04	1.44
7 wks jungle 4 wks outside, Group A	205	15	19.9	1.17	1.41
7 wks outside, 4 wks jungle, Group B	205	15	19.9	1.08	1.32

* Reference ARL Report on Project No. 2 (2-7, 11, 13, 15, 17, 19) dated 18 October 1943.

** These tables will be found at the end of this section.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that this is crucial for the company's financial health and for providing reliable information to stakeholders.

2. The second part outlines the specific procedures for recording transactions. It details the steps from initial entry to final review, ensuring that all data is captured correctly and consistently.

3. The third part addresses the role of the accounting department in overseeing these processes. It highlights the need for regular audits and the implementation of internal controls to prevent errors and fraud.

4. The fourth part discusses the importance of transparency and communication. It stresses that all employees should be aware of the financial policies and procedures, and that there should be open channels for reporting any issues or concerns.

5. The fifth part covers the legal and regulatory requirements that the company must adhere to. It mentions the various laws and standards that govern financial reporting and the consequences of non-compliance.

6. The sixth part provides a summary of the key points discussed in the document. It reiterates the importance of accuracy, transparency, and compliance, and encourages all employees to take responsibility for their actions.

7. The seventh part concludes the document with a statement of intent. It expresses the company's commitment to maintaining high standards of financial integrity and to providing the best possible service to its customers.

8. The eighth part includes a list of references and sources used in the document. It provides a comprehensive list of books, articles, and other materials that were consulted during the research process.

9. The ninth part contains a list of appendices. These include additional documents, charts, and tables that provide further detail on the topics discussed in the main text.

10. The tenth part is a final section that provides contact information for the authors and for the company. It includes phone numbers, email addresses, and a website where more information can be found.

MEAN₆ PLASMA ATABRINE CONCENTRATIONS, JUNGLE GROUPS

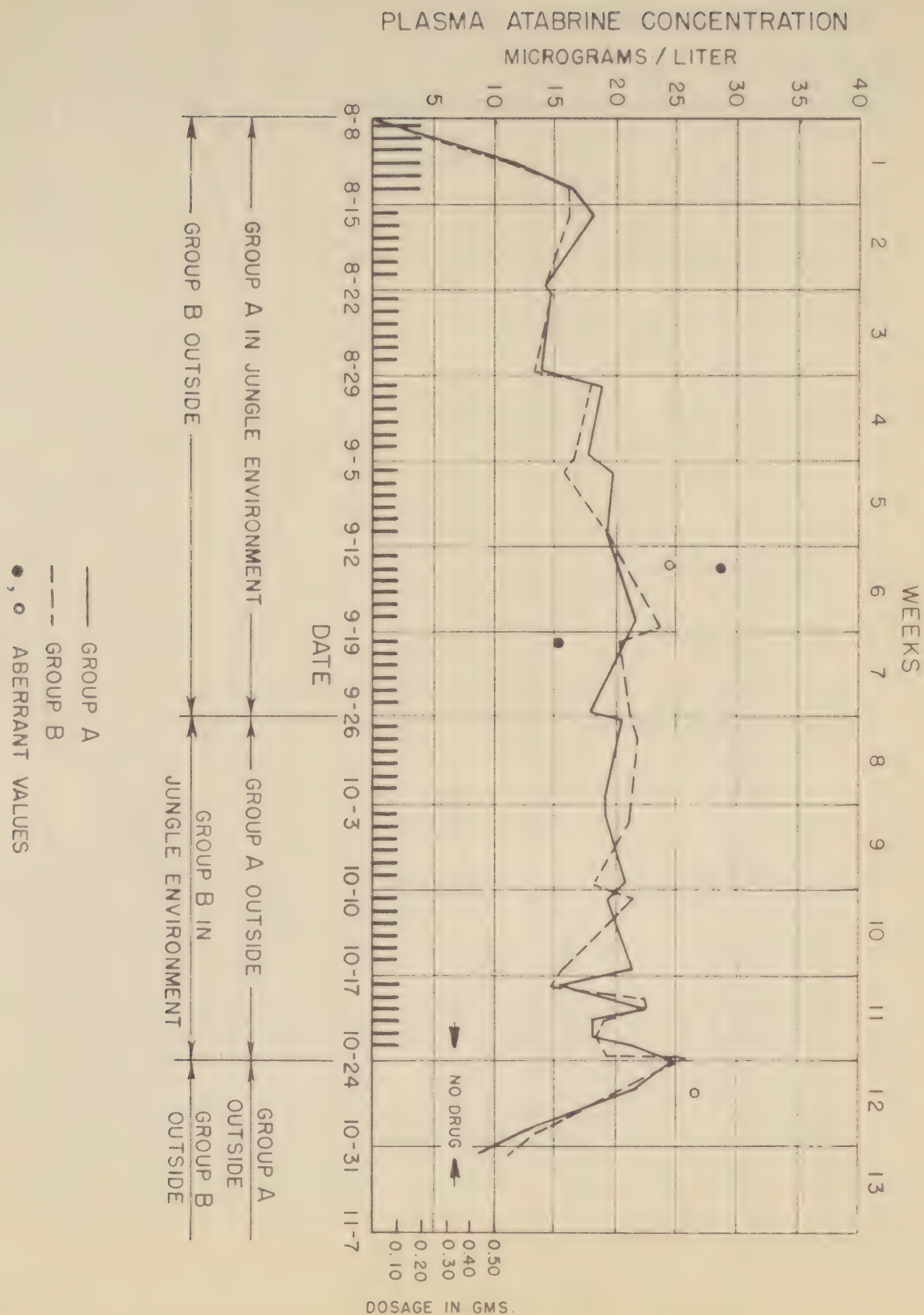
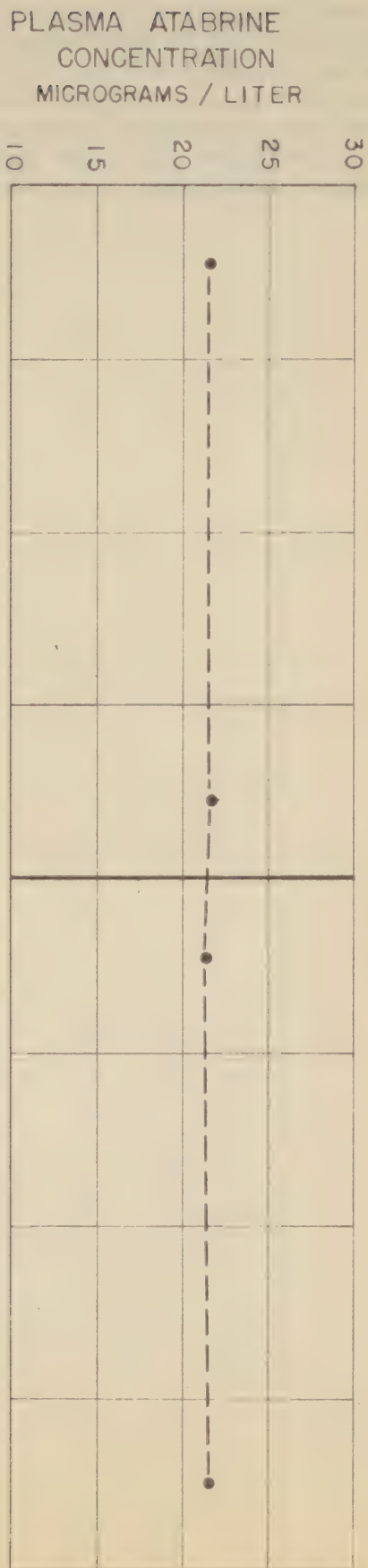


CHART - 24

HIGH RATE OF SWEAT LOSS IN JUNGLE DOES NOT AFFECT PLASMA

ATABRINE CONCENTRATION



SWEAT LOSS DURING WORK
GRAMS PER 5 WORK PERIODS

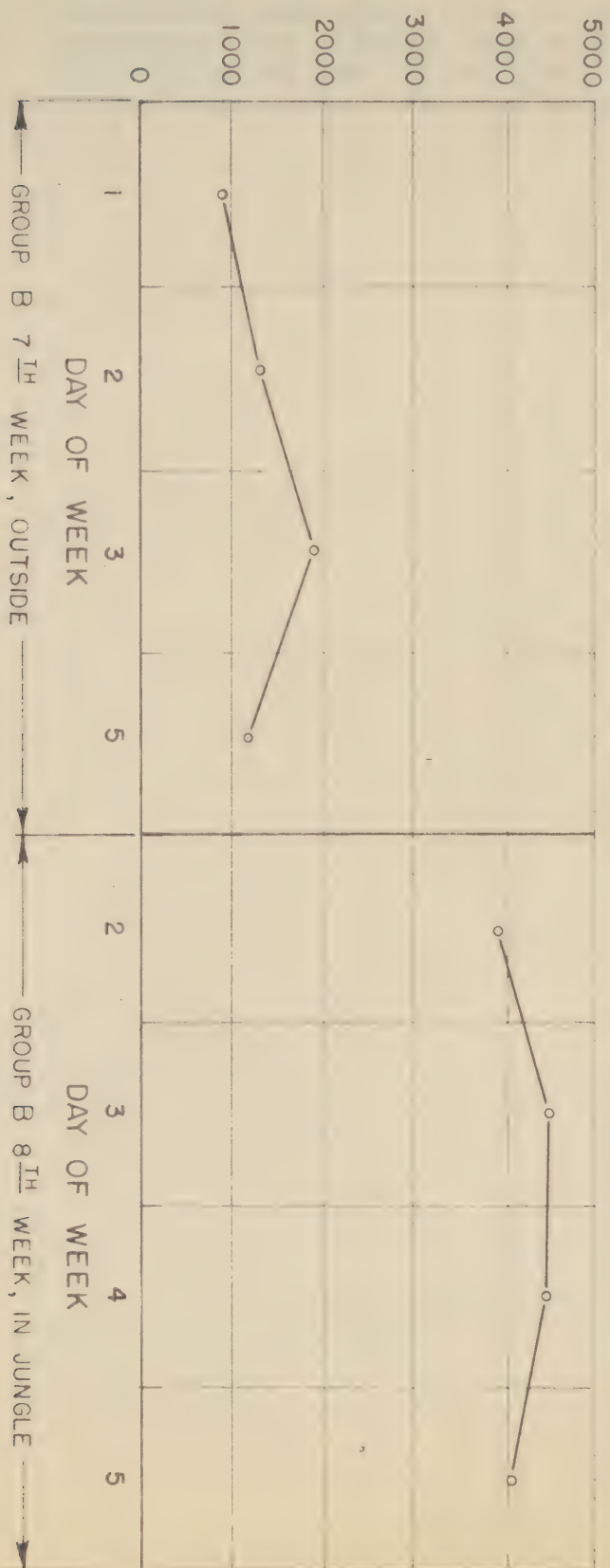


CHART - 25

CHART - 25

The mean levels for the three groups are approximately the same as is the degree of individual variability as measured by the standard deviations. Both jungle groups had their low and high men and others exhibiting breakaways. It is concluded, therefore, that the hot, humid environment has no effect upon the process of absorption and storage of atabrine and that the required dosage schedule for effective suppressive therapy is not influenced by the climatic conditions studied.

BLEEDING H ₁						
WEEK	68% RANGE of MEAN ^G			DISPERSION		MEAN ^A
Number	Date	NO. of men	MEAN ^G	RANGE of MEAN ^G	Std	RANGE
1	Aug. 12	15	11.6	11.0-12.1	1.21	12.1-13.9
2	16	13	18.2	17.5-19.0	1.17	15.6-21.3
3	23	15	14.6	13.9-15.4	1.22	11.9-17.8
4	30	15	13.7	18.0-19.4	1.15	16.2-21.5
5	Sept 8	15	19.7	18.2-21.5	1.39	14.2-27.4
6	13	15	28.6	26.0-31.5	1.45	19.8-41.4
7	20	14	16.0	14.5-17.7	1.46	11.0-23.4
8	27	15	20.1	18.0-22.5	1.55	13.0-31.2
9	Oct 4	15	19.1	17.4-21.0	1.44	13.2-27.5
10	11	15	19.3	17.9-20.8	1.34	14.4-25.8
11	18	15	15.2	13.9-16.7	1.42	10.8-21.5
12	25	14	20.6	19.0-22.2	1.33	15.4-27.1

BLEEDING H₂ + 5

WEEK		NO. of MEN	MEAN ^G	68% RANGE of MEAN ^G	DISPERSION		MEAN ^A
Number	Date				<i>σ</i>	68% RANGE	
1	Aug 14	15	25.2	23.9-26.6	1.23	20.6-30.9	25.7
2	21	15	22.6	21.8-23.4	1.15	19.6-26.0	22.8
3	28	15	20.8	19.7-21.8	1.23	16.9-25.4	21.3
4	Sept 4	15	26.7	25.2-28.4	1.26	21.3-33.6	27.5
5	11	15	34.8	32.3-37.6	1.34	26.0-46.7	36.4
6	18	15	36.6	33.7-39.8	1.37	26.7-50.4	38.7
8	Oct 2	15	28.1	26.1-30.2	1.33	21.0-37.3	29.3
9	9	15	22.8	21.0-27.7	1.37	16.6-31.2	23.9
10	16	14	24.0	22.1-26.2	1.38	17.4-33.2	25.2

BLEEDING H ₂							
WEEK	Number	Date	NO. of MEN	MEAN ^G	68% RANGE of MEAN ^G	DISPERSION	MEAN ^A
	Aug.					68% RANGE	
1	14	15	16.2	15.4-17.0	1.21	13.3-19.6	16.4
2	21	14	14.3	13.7-15.0	1.18	12.2-16.9	14.5
3	28 Sept 4	15	13.6	12.9-14.4	1.25	10.9-17.1	13.9
4	5	15	17.7	16.2-19.2	1.39	12.7-24.6	18.6
5	11	15	19.4	18.1-20.8	1.48	13.1-26.8	21.1
6	18	15	21.4	19.6-23.1	1.40	15.3-30.0	23.9
7	25	15	17.9	16.1-19.9	1.49	12.0-26.4	17.5
7	26 Oct 2	15	20.8	19.1-22.2	1.42	14.7-27.7	22.3
8	9	15	19.2	17.1-21.6	1.57	12.3-30.1	21.1
9	16	15	20.7	19.5-22.0	1.42	14.5-20.1	22.0
10	19	14	21.3	19.6-23.1	1.38	15.4-29.4	22.7
11	20	14	20.1	18.8-21.5	1.29	15.6-25.8	20.7
11	21	14	22.1	20.1-24.3	1.42	15.5-31.5	23.6
11	22	14	18.0	16.4-19.7	1.41	12.7-25.3	19.1
11	23	14	18.2	16.8-19.8	1.37	13.3-24.9	19.0
11	24	14	21.2	19.7-22.8	1.31	16.1-27.9	22.0
12	27	14	24.4	22.7-26.1	1.30	18.7-31.8	25.2
12	29	13	21.4	19.9-23.0	1.31	16.4-28.0	22.1
12	Nov 1	13	12.7	11.4-14.1	1.47	8.7-18.6	13.5
13	7	14	8.7	7.9-9.6	1.42	6.1-12.3	9.2

MEAN^G AND CHARACTERISTICS OF WEEKLY

PLASMA ATABRINE LEVELS

JUNGLE GROUP A

0.6 gm/wk

BLEEDING H₁

WEEK	Number	Date	No. of MIN	MEAN ⁰	68% RANGE of MEAN ⁰	DISPERSION		MEAN ^A
						68	68% RANGE	
1	Aug 12	15	15	11.2	10.5-12.1	1.32	8.5-14.8	11.6
2	16	14	14	16.1	15.2-17.1	1.25	12.9-20.2	16.5
3	23	15	15	14.9	14.4-15.4	1.13	13.1-16.9	15.0
4	30	14	14	17.9	17.3-18.6	1.23	14.6-22.0	18.2
5	Sept 6	15	15	15.7	14.6-17.0	1.38	11.7-21.2	16.5
6	15	15	15	25.2	23.7-26.6	1.08	19.8-32.0	25.8
7	20	15	15	20.4	18.7-22.2	1.39	14.7-28.2	21.3
8	27	14	14	21.6	20.3-23.1	1.27	17.1-27.4	22.2
9	Oct 4	15	15	21.1	19.8-22.5	1.28	16.4-27.1	21.7
10	11	15	15	21.4	20.1-22.9	1.30	16.4-28.0	22.3
11	18	14	14	14.6	13.6-15.7	1.30	11.2-19.1	15.0
12	25	14	14	26.6	21.5-23.8	1.21	18.6-27.4	23.0

BLEEDING H₂ + 5

WEEK	Number	Date	No. of MIN	MEAN ⁰	68% RANGE of MEAN ⁰	DISPERSION		MEAN ^A
						68	68% RANGE	
1	Aug 14	15	15	25.6	24.3-26.9	1.22	21.0-31.1	26.1
2	21	15	15	20.1	19.4-20.7	1.14	17.6-22.8	20.2
3	28	15	15	19.2	18.4-20.0	1.17	16.4-22.4	19.4
4	Sept 4	15	15	24.2	23.2-25.2	1.17	20.7-28.2	24.5
5	11	14	14	29.7	28.5-31.0	1.17	25.4-34.7	30.4
6	18	15	15	33.5	31.2-35.9	1.31	25.6-43.8	34.6
8	Oct 2	15	15	26.8	25.0-28.6	1.30	20.6-34.8	27.6
9	9	10	10	22.7	21.9-24.1	1.21	18.8-27.5	23.1
10	15	15	15	22.3	21.3-23.3	1.19	18.8-26.5	22.7

BLEEDING H₂

WEEK	Number	Date	No. of MIN	MEAN ⁰	68% RANGE of MEAN ⁰	DISPERSION		MEAN ^A
						68	68% RANGE	
1	Aug 14	15	15	16.0	15.1-17.0	1.26	12.7-20.2	16.4
2	21	14	14	14.3	13.7-14.9	1.16	12.3-16.6	14.4
3	28	15	15	13.4	12.6-14.2	1.26	10.6-16.9	13.7
4	Sept 4	15	15	16.6	14.9-18.4	1.50	11.0-24.9	16.8
5	11	15	15	19.6	18.5-20.9	1.27	15.5-25.0	20.2
6	18	15	15	24.0	22.5-25.7	1.29	18.7-30.9	24.7
7	25	15	15	21.0	19.3-22.9	1.39	15.1-29.3	22.1
7	26	15	15	21.5	19.9-23.3	1.36	15.9-29.2	22.5
8	Oct 2	15	15	21.2	19.9-22.5	1.27	16.6-26.9	21.7
9	9	14	14	18.4	17.2-19.7	1.28	14.3-23.6	18.9
10	16	13	13	15.6	14.5-16.8	1.31	11.9-20.4	16.2
11	19	15	15	22.2	21.0-23.3	1.22	18.1-27.1	22.6
11	20	15	15	22.6	21.3-23.9	1.25	18.0-28.2	23.1
11	21	15	15	19.1	18.1-20.2	1.23	15.5-23.5	19.5
11	22	15	15	17.8	16.4-19.3	1.35	13.0-24.3	22.1
11	23	15	15	19.4	18.3-20.6	1.27	15.4-24.6	19.9
11	24	15	15	26.0	24.5-27.5	1.25	20.8-32.4	26.5
12	27	13	13	25.9	24.1-27.8	1.29	20.0-33.5	26.6
12	29	14	14	13.7	12.8-14.6	1.28	10.7-17.5	14.1
13	Nov 1	14	14	11.1	10.1-12.2	1.14	7.7-16.0	11.7

MEAN AND CHARACTERISTICS

OF WEEKLY PLASMA

ATABRINE LEVELS

FOR

JUNGLE GROUP B

0.6 gm/wk

SECTION IV - Special Studies

1. Absorption of atabrine following a single dose and effect of "booster" doses upon time to reach equilibrium.a. Procedure.

- (1) Subjects. In order to obtain further information on the behavior of atabrine, with respect to its rate of absorption and removal from plasma in relation to dosage, certain special studies were carried out employing an additional group of 14 healthy, young (18 to 20 years) volunteers. These special studies began in mid-summer and continued into mid-fall (for environment see Chart 15, Section II and Chart 22, Section III. Throughout the entire period these men engaged in regular company duty.
- (2) Grouping by dosage schedule - These subjects were divided into three groups for study of absorption under different dosage schedules. Groups C-1 and C-2, of four men each, were put under the same basic schedule of single daily doses of 0.2 gm for 17 out of the first 19 and 20 days for the two groups respectively, and 0.1 gms, 6 days a week, for the remainder of the study (10 weeks for C-1 and 9 weeks for C-2). These and other minor differences were considered not sufficiently great to prevent combining the results obtained for these two groups. Dosage schedule for group C-3 consisted in single daily doses of 0.3 grams for six days during the first week, and daily doses of 0.2 grams for six days during the second week; for the next seven weeks the daily maintenance dose of 0.1 gram, six days per week, was administered. Thus, after higher dosage schedules during the first three weeks, all three groups were subjected to the same maintenance schedule for the remainder of the study.
- (3) Blood sampling - Blood samples (30 ml) for determination of absorption curves were taken before the drug was given and 2, 4, 6, 8, 12, 24, and at times 48 hours after the dose. In addition, samples were obtained regularly three times weekly in accordance with the schedule already described (H_1 , H_2 , $H_2 + 5$).

b. Results.

- (1) Absorption. The rise and fall of plasma atabrine concentration immediately following the administration of a single dose is shown in Charts 26 and 27 (data in Table 15)*

* Tables will be found at end of Section.

THE HISTORY OF THE UNITED STATES

The history of the United States is a story of growth and change. It begins with the first settlers and continues through the present day. The story is one of a nation that has grown from a small colony to a great power.

CHAPTER I

The first settlers of the United States were the Pilgrims. They came to the New World in 1620 and founded the Plymouth colony. They were followed by other settlers, and the colonies grew in number and size. The Pilgrims were a group of English Puritans who sought religious freedom.

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CHART-10

POST ABSORPTION CURVES OF PLASMA ATABRINE CONCENTRATION FOLLOWING 0.2 GM DOSE

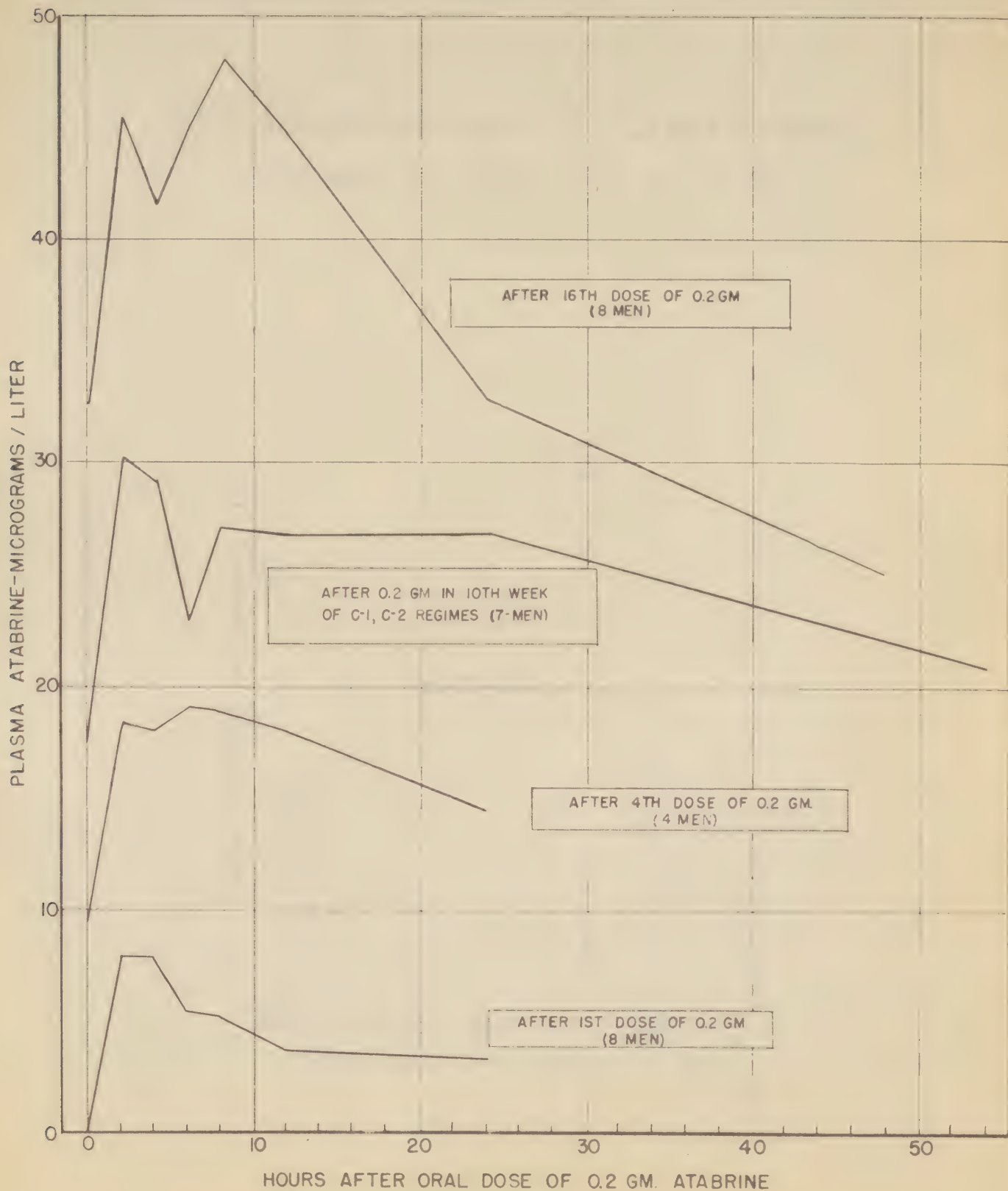
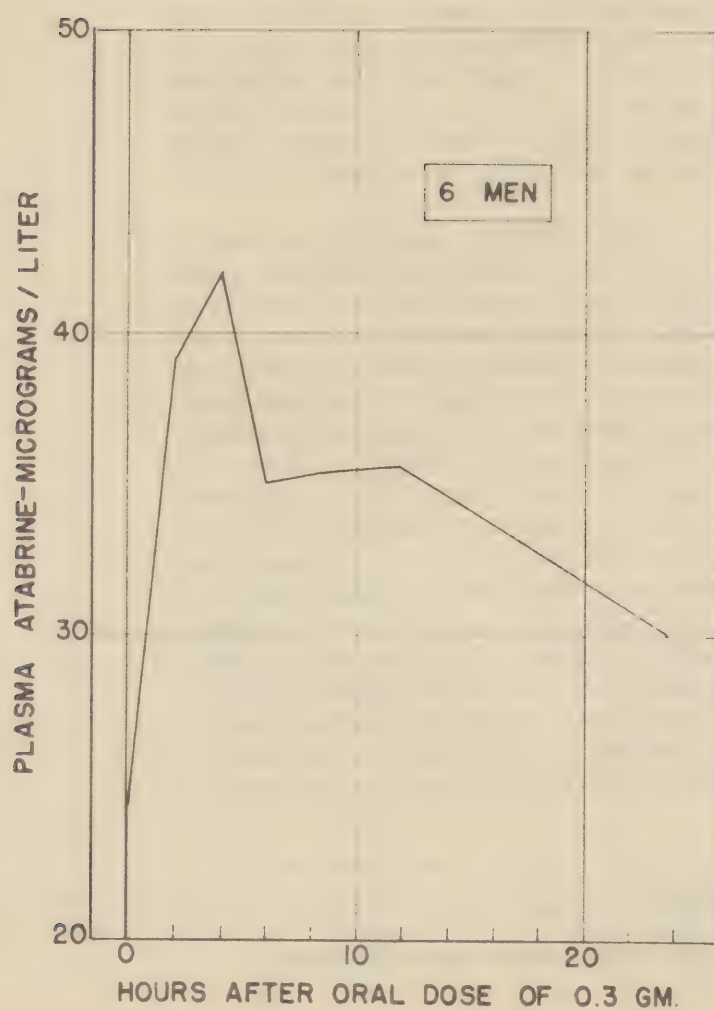


CHART- 27

POST ABSORPTION CURVE OF PLASMA ATABRINE
FOLLOWING 4TH DAILY DOSE OF 0.3 GM



for subjects who had previously received varying amounts of the drug, from none up to a sufficient quantity to have established equilibrium. The absorption curves have the same general shape, although the absolute plasma levels resulting from a given dose vary, depending upon the previous atabrine dosage. Absorption from the gastrointestinal tract is rapid. A major portion of the rise in concentration takes place within 2 hours and the peak level is reached within 8 hours. Thereafter there is a decline in concentration. In 24 hours the level is reduced to a point which is above the pre-dose level, the net gain in concentration for a given dose depending upon status of the subjects with respect to previous intake of the drug. If no dose is given after one day, the concentration continues to decline. With a single dose of 0.3 gm (Chart 27) except for the greater increase in level induced by the larger dose, the results were similar to those following the 0.2 dose. Some of the curves are bimodal in shape, with two peak values which are believed to characterize the mechanism of absorption. A more extensive consideration of these curves, from the standpoint of their significance with respect to the buildup of suppressive and therapeutic atabrine levels is given in Appendix B.

- (2) Effect of initial booster dose. It was shown that the mean equilibrium plasma atabrine level is directly proportional to the dosage; and that the time required to reach an equilibrium state is independent of dosage provided the weekly dosage schedule remains constant. Furthermore, it has been demonstrated that the underlying plasma level at any time when expressed as a percentage of the final equilibrium level, is the same regardless of dosage. It follows directly from these statements that the time required to reach a desired plasma level can be shortened if, at the outset, the drug is administered at a dosage rate higher than that required to maintain the desired level in equilibrium. An example will make this clear. The rate of rise in plasma level in any week is approximately 50% of the difference between the level already established and the final equilibrium level. Thus at dosages of 0.6 and 1.2 gm/wk the predicted plasma levels at the end of the first and subsequent weeks will be:

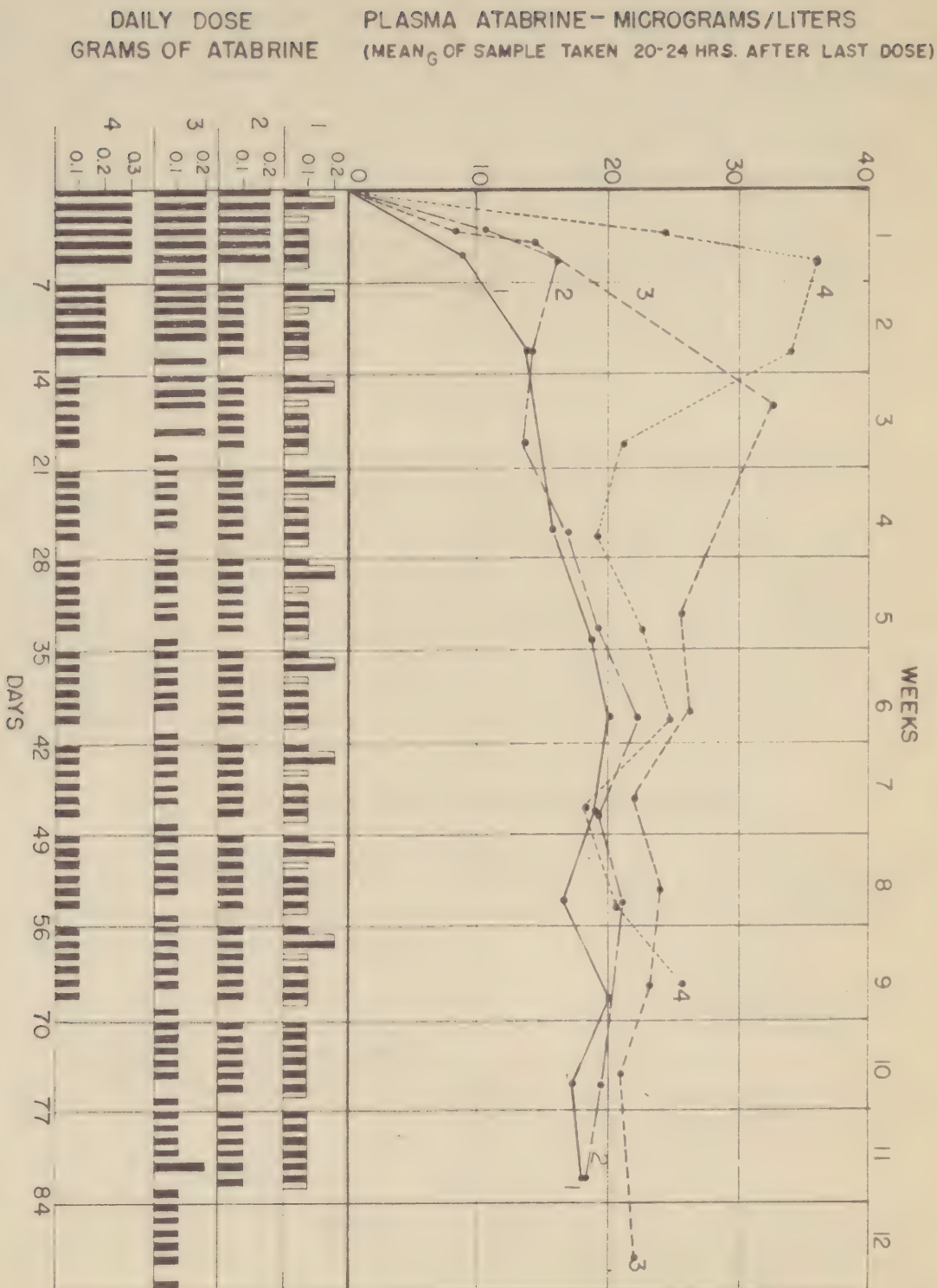
Week	Plasma Concentration	
	0.6 gm/wk	1.2 gm/wk
1	9.0	18.0
2	13.5	27.0
3	15.7	31.5
4	16.9	33.7
5	17.4	34.8
Equilibrium	18.0	36.0

At the higher dosage rate of 1.2 gm/wk the plasma level at the end of the first week is as high, according to this theoretical relationship, as is the equilibrium level ultimately attained by the 0.6 gm dosage. If, therefore, the dosage during the first week only is doubled as compared with the subsequent maintenance dosage, the plasma concentration will be raised to the desired level within this period rather than in 5 weeks or more, which is required without the initial booster dose. This conclusion is partially borne out by the experimental evidence as shown in Chart 28, in which are compared the plasma atabrine levels in relation to time for the two groups (C-1 and 2, C-3) on the same maintenance dosage rate but with differing dosage schedules during the first two weeks. For comparison, the curve of mean plasma level for the ARTC group on the constant 0.6 gm/wk schedule is shown. Also plotted on the chart is the curve for the jungle group, which received an initial booster dose of 1.2 gm/wk for the first week. In the C-1 and 2 and C-3 groups, plasma concentrations in excess of the ultimate maintenance level were reached within a week. It will be noted that the time required to reach the actual equilibrium state was not changed but it was approached by a declining concentration curve from higher levels in contrast to the progressively rising curves for the group receiving no booster dose. The jungle group, receiving the double dose for the first week only, did not follow the theoretical prediction exactly, showing a decline during the second week and close agreement with the ARTC group thereafter. It is to be noted that the equilibrium levels finally attained on the maintenance regimen of 0.6 gm/wk did not differ significantly among the four groups. The practical significance of the initial high dosage schedule is great since the time required to reach an effective suppressive level may be an important factor in the planning of troop movements into malarious areas. The regular inclusion of the initial booster dose in the schedule of administration of atabrine is therefore recommended.

2. Rates of buildup and dieaway of plasma atabrine level.

a. Procedure. At the end of the eleventh week both jungle groups A and B were allowed to work out-of-doors and administration of the drug was discontinued in order to study the rate of fall of plasma atabrine level (dieaway). The subsequent rise of concentration on restoration of

PLASMA ATABRINE LEVELS OBTAINED WITH DIFFERENT PRIMING DOSES MAINTENANCE DOSAGE, 0.6 GM. / WEEK



suppressive atabrine therapy (buildup) was also determined. Half of these men were without drug for one week and the other half for two weeks. In order to insure comparability of the two dieaway curves, groups A and B were rearranged into two new groups (X and Y) which were comparable with respect to: duration of previous heat exposure, time since removal from the jungle environment, weight of men, plasma atabrine level over the four week period before dieaway, and atabrine levels after six days without drug. Atabrine suppressive therapy was resumed in group Y after an interval of one week and in group X after two weeks without drug (See Chart 29). The means and dispersion of the data are recorded in Table 16 and the crude data will be found in Appendix K. To obtain additional dieaway data on a large group, all of Company C, AITC group (84 men) were similarly studied. Company C, Section 1 (0.4 gm/wk) took their last dose on November 12, 1943; Company C, Section 2 (0.6 gm/wk) took their last dose on November 11, 1943; both were blood thereafter on November 13, 15, 17 and 20. Chart 30 compares the dieaway curves on the two dosage regimens. No buildup studies were made on this group. (data in Table 16a)

b. Results. The mechanisms which determine the rate of increase of plasma level under a constant dosage schedule and the rate of decrease from an equilibrium level after discontinuing the drug are considered theoretically in Appendix B and the values predicted from the theory are compared with the observed concentrations. The rate of dieaway amounts to approximately 10% per day resulting in a drop of about one-half in a week. Restoration of the dosage regimen results in increasing plasma levels which approach the equilibrium level at the same percentage rate as obtains with the original administration of the drug, namely, 50% per week of the difference in plasma level at the beginning of the week and the equilibrium level (See Chart 29).

3. Plasma levels obtained with therapeutic doses.

a. Procedure. At the beginning of the 12th week the two sections of Company C, AITC Group, after having developed equilibrium levels on their respective dosage schedules of 0.4 and 0.6 gm/wk, were placed upon a modified therapeutic regimen, as follows:

1st day - 0.5 gm (0.1 gm at breakfast and 0.2 gm at lunch and supper)
Next 5 days - 0.3 gm (0.1 gm at breakfast, lunch and supper)
7th day - 0.1 gm at breakfast

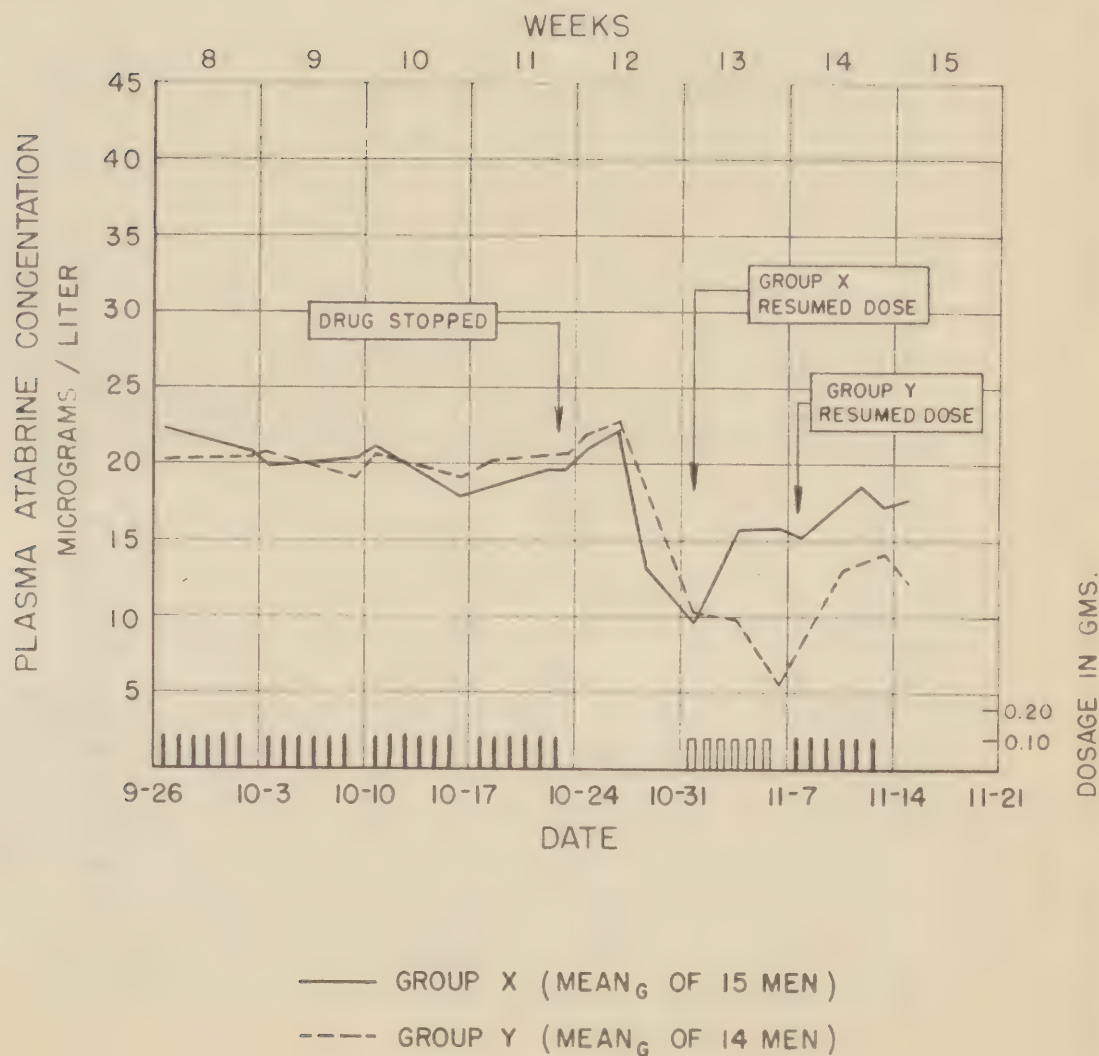
Blood samples were taken before the noon meal on the 4th, 5th and 6th days.

b. Results.

- (1) The increases in plasma levels, as shown in Chart 31, for the two sections at the end of the period were approximately the same (above their previously established equilibrium levels, 30.8 and 30.5 micrograms/L respectively). These changes in concentration with the institution of the new dosage regimen were found to follow the same basic law of buildup as had previously determined the increase toward equilibrium on the lower suppressive regimen and

CHART - 29

INFLUENCE OF INTERRUPTION OF DOSAGE ON PLASMA ATABRINE LEVELS (0.6 GM. / WEEK SCHEDULE, 30 MEN)



MEAN₆ PLASMA ATABRINE LEVELS OF TWO GROUPS OF MEN FOR ONE WEEK AFTER DISCONTINUING SUPPRESSIVE THERAPY

DIE AWAY CURVE FOR GROUP THAT RECEIVED
0.4 GRAMS PER WEEK FOR ELEVEN WEEKS

DIE AWAY CURVE FOR GROUP THAT RECEIVED
0.6 GRAMS PER WEEK FOR ELEVEN WEEKS

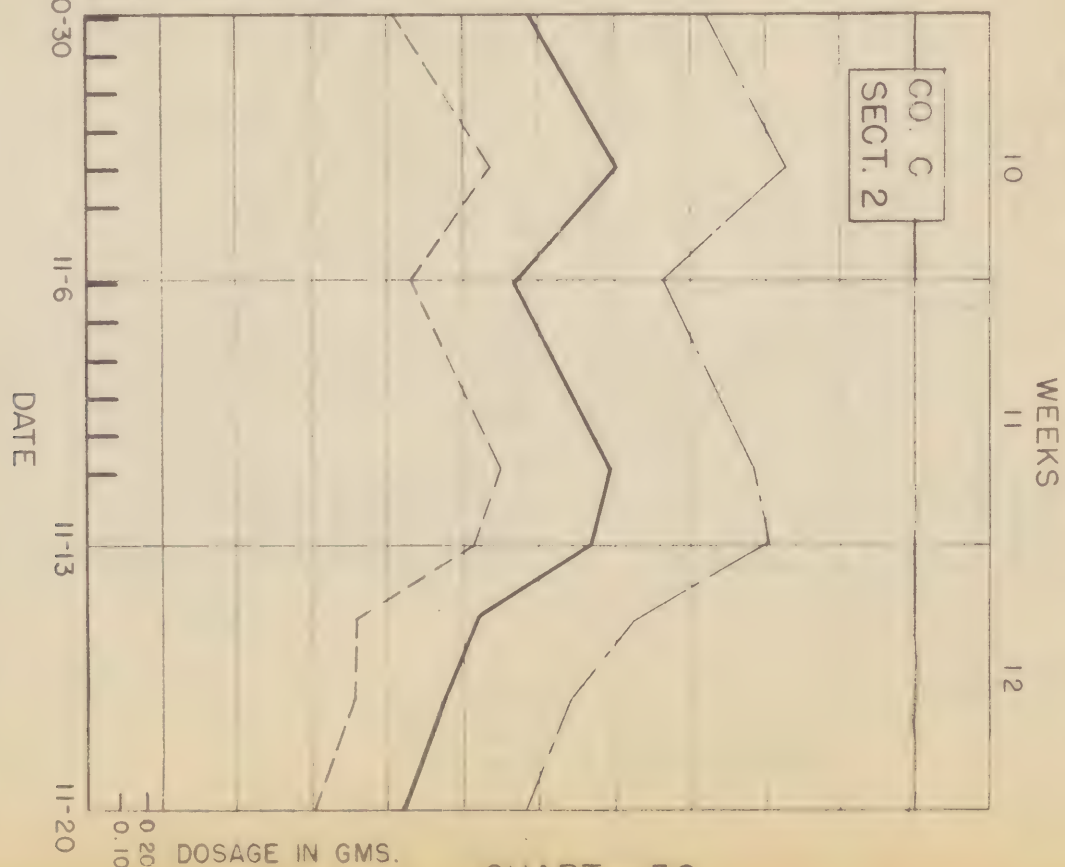


CHART - 30

MEAN₆ X SD } 68 % RANGE
MEAN ± SD

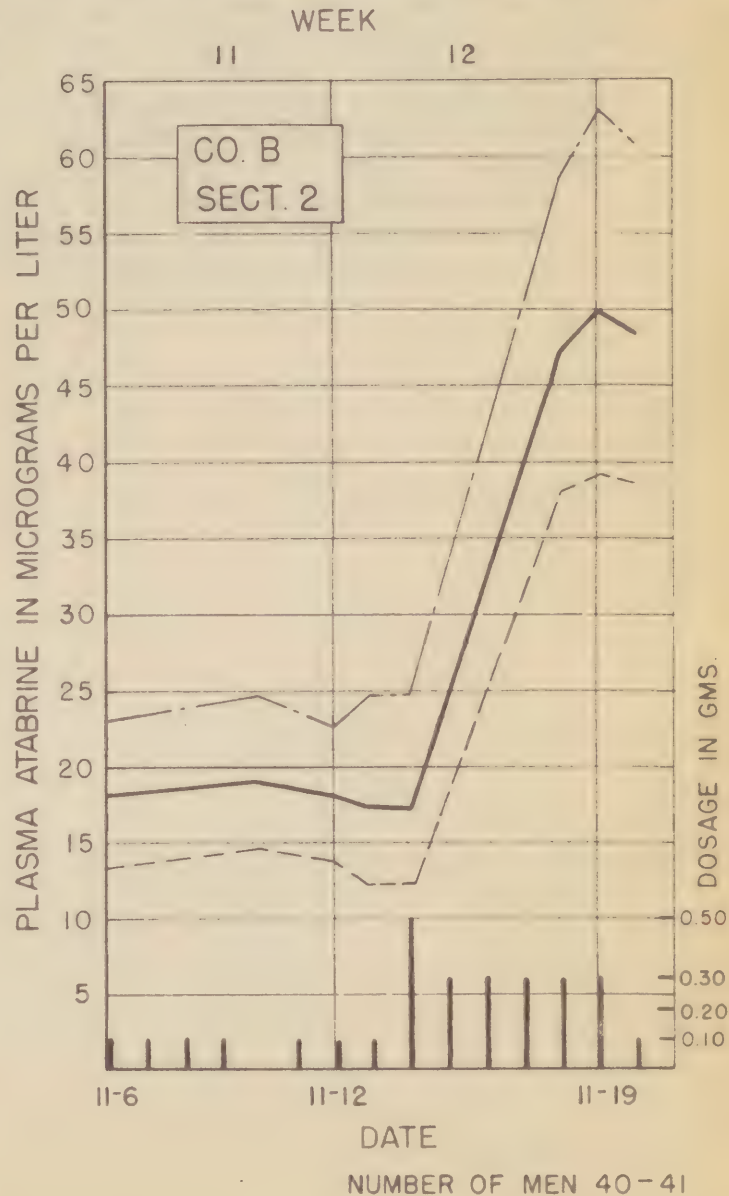
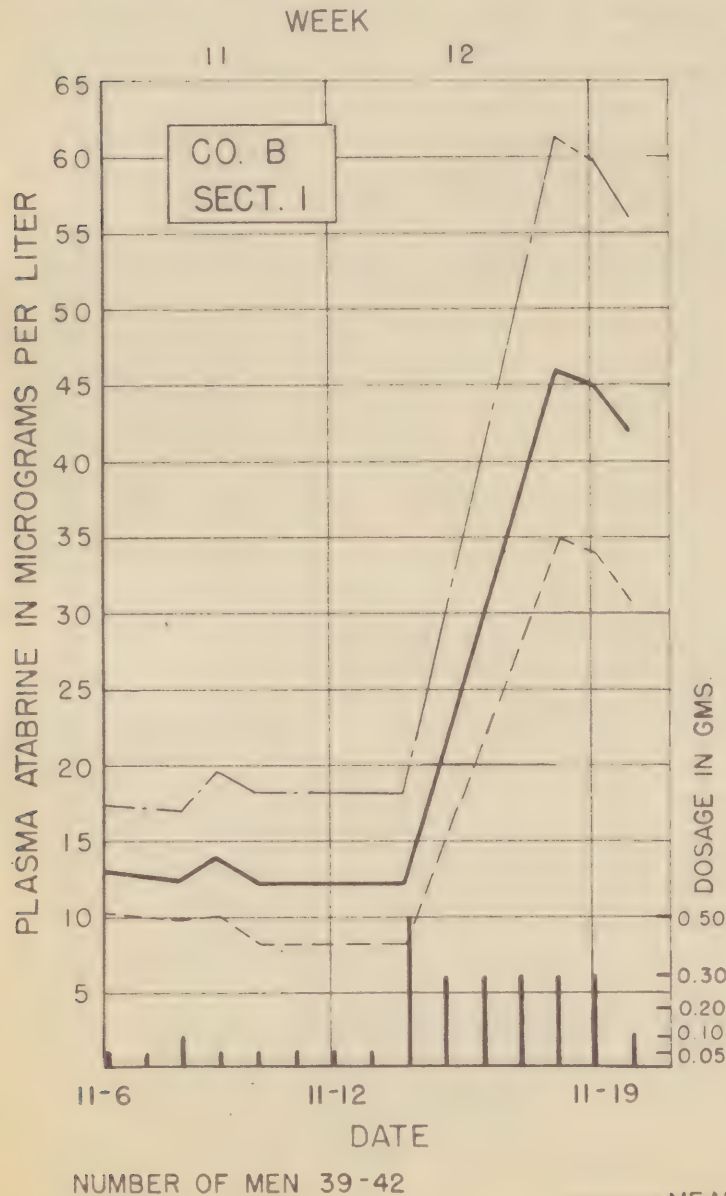
CHART - 30

CHART - 31

MEAN_G PLASMA ATABRINE LEVELS OF TWO GROUPS OF MEN RECEIVING THERAPEUTIC DOSAGE FOR ONE WEEK

THERAPEUTIC DOSAGE AFTER RECEIVING
0.4 GRAMS PER WEEK FOR ELEVEN WEEKS

THERAPEUTIC DOSAGE AFTER RECEIVING
0.6 GRAMS PER WEEK FOR ELEVEN WEEKS



there was close agreement between the levels predicted by the theoretical equation and the observed values.

- (2) An important objective of this phase of the study was to determine whether or not the men with persisting low and high plasma levels while on suppressive therapy would continue in the same relation to the group mean under the subsequent therapeutic regimens. The correlations are shown in Charts 32 and 33. Chart 32 shows the distribution with respect to the equilibrium levels attained under the two suppressive regimens and the subsequent distribution of these low, central and high men after receiving therapeutic doses as outlined above. No great shift in distribution will be noted, the majority of the subjects remaining in their original categories under the therapeutic regimen. The correlation is shown in another way in Chart 33 where therapeutic level is plotted against suppressive level. A definite relationship is evident but the correlation is not high. One cannot, therefore, predict with any certainty, the relative therapeutic level for an individual in a group from his relative suppressive level except in broad categories.

RELATIONSHIP BETWEEN LEVEL ATTAINED ON SUPPRESSIVE THERAPY AND LEVEL REACHED ON THERAPEUTIC DOSES

COMPANY B
SECTION II
(42 MEN)

0.6 GRAMS PER WEEK 2.1 GRAMS PER WEEK

COMPANY B
SECTION I
(39 MEN)

0.4 GRAMS PER WEEK 2.1 GRAMS PER WEEK

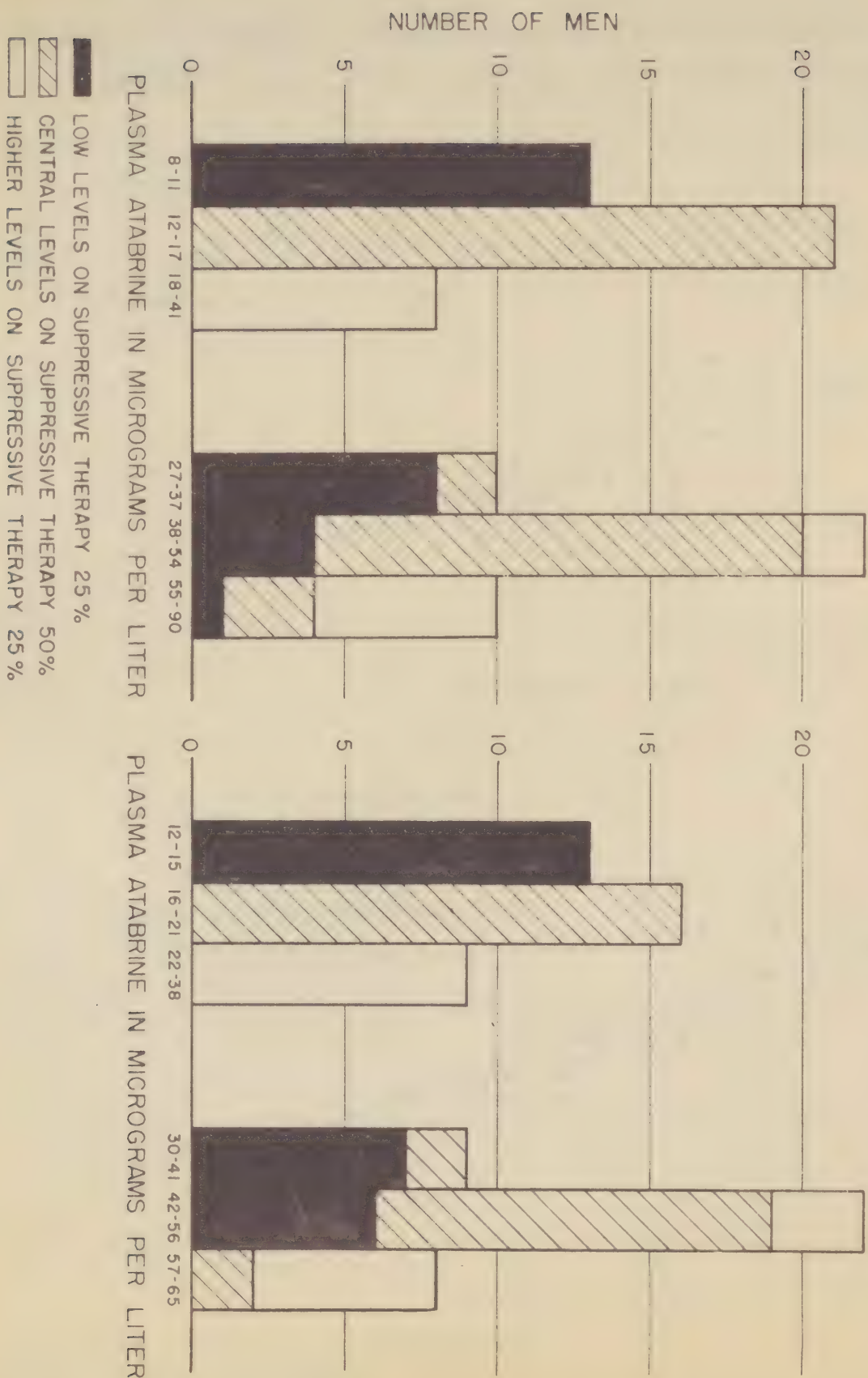
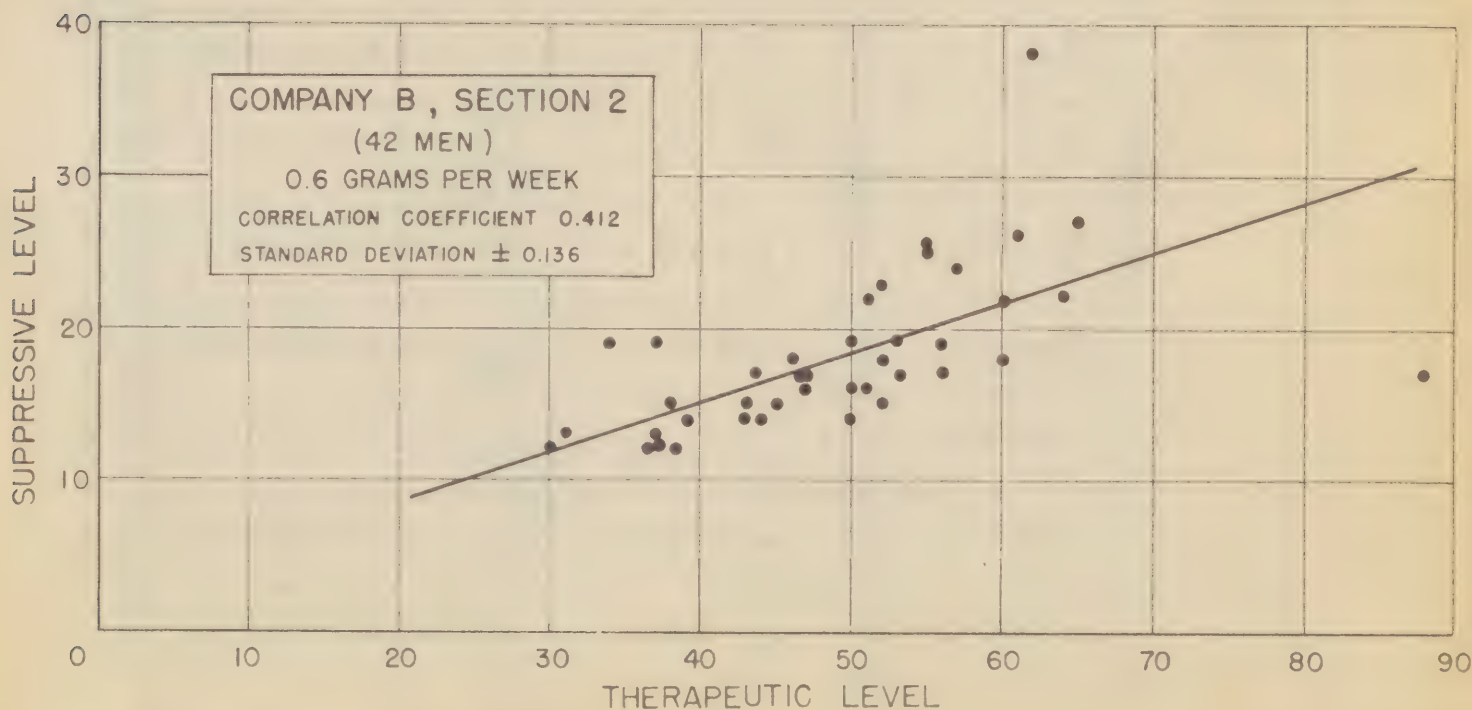
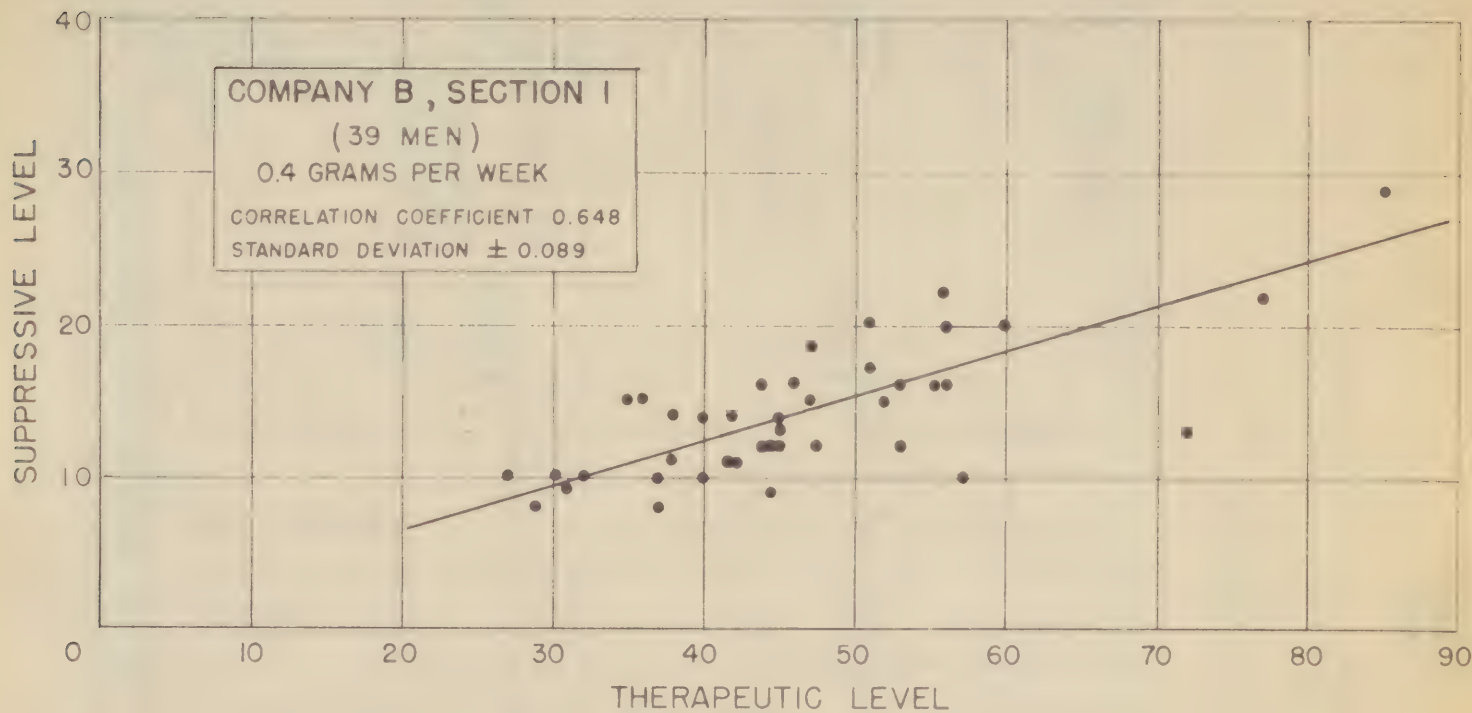


CHART - 32

CHART - 33

RELATIONSHIP BETWEEN PLASMA ATABRINE LEVEL ATTAINED ON SUPPRESSIVE THERAPY AND LEVEL REACHED ON THERAPEUTIC DOSES



BOTH GROUPS RECEIVED 2.1 GRAMS PER WEEK
ON THE THERAPEUTIC REGIMEN

TAB. 15
Sheet 1

PLASMA ATAGRINE (Micrograms per Liter)
GROUP C

See sheet 4 for explanation of dosage schedules.

[illegible]

* The men in Group C2 received by mistake 0.1 gm of atabrine shortly after the 8 hour bleeding. Correction of the total dosage was made by a corresponding reduction the following day.

TABIE 15
Sheet 2

Micrograms per Liter)
GROUP C

See Sheet 4 for explanation
of dosage schedules.

[illegible]

TABLE 15
Sheet 3

GROUP C

See sheet 4 for explanation of dosage schedules.

[illegible]

TABLE 15
Sheet 4

PLASMA ATABRINE (Micrograms per liter)

See sheet 4 for explanation
of dosage schedules.

C1	Date	10/30	10/30						10/31	10/30		11/1	11/6	11/8	11/13	11/15
	Day of Experiment	74	74						75	74		76	81	83	88	90
	Hours After Drug	20-0	2	4	6	8	12	24		20		54	20	53	20	53
Jones Gervais Peterson Hudson		16	30	-	24	29	28	-		16		19	18	19	18	18
		24	34	35	27	31	31	33		24		28	29	29	24	26
		22	39	35	29	33	41	36		22		29	36	32	31	11
		17	-	-	21	25	25	22		17		20	23	18	18	19
MEAN		19.5	34.1	35.0	25.1	29.4	30.7	29.7		19.5		23.6	25.6	23.7	22.2	17.7
C2	Date	10/30	10/30						10/31	11/1	11/6	11/6	11/8	11/13	11/15	
	Day of Experiment	68	68						69	70	75	75	77	82	84	
	Hours After Drug	20-0	2	4	6	8	12	24	54	20	5	53	20	53		
Clifford Hedburg Brown Mills		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		17	28	31	22	26	28	29	19	25	23	19	22	23	22	
		19	34	-	26	31	25	31	23	30	31	23	21	22	22	
		11	20	19	14	18	16	16	13	15	18	14	13	12		
MEAN		15.2	26.7	24.3	20.0	24.4	22.4	24.3	17.8	22.4	23.4	18.3	18.2	18.2		
MEAN (C1 & C2)		17.5	30.2	29.2	22.8	27.1	26.8	26.9	-	20.7	-	21.1	22.1	21.2		

Groups C1 and C2:- Followed for 91 and 85 days. The men in these groups received 17 priming doses of 0.2 gm per day from day 0 to day 18 (C1) and day 0 to day 19 (C2). Thereafter, each man received 6 doses of 0.1 gm each week. Post absorption curves were followed after the 1st, 4th and 16th doses of 0.2 gm; on 10-30 (day 74 and day 68) a post absorption curve was run after a dose of 0.2 gm.

Group C3:- Followed for 64 days. Received 6 priming doses of 0.3 gm per day during the 1st week; 6 doses of 0.2 gm per day during the 2nd week. Thereafter, each man received 6 doses of 0.1 gm each week. The post absorption curve was followed after the 4th dose of 0.3 gm.

ALL FINDINGS GROUP X

WEEK	Number	Date	No. of MEN	MEAN ⁵	68% RANGE of MEAN ⁵	DISPERSION		MEAN ¹
							68% RANGE	
8	Sept 27	14	14	22.2	19.9-24.8	1.51	14.8-33.5	24.1
8	Oct 2	15	15	20.7	19.0-22.6	1.40	14.7-29.0	22.0
9	4	15	15	19.9	18.0-21.9	1.46	13.7-29.0	21.5
9	9	14	14	20.3	17.3-22.1	1.45	16.6-34.8	21.8
10	11	15	15	21.1	19.4-23.0	1.39	15.2-29.4	22.3
10	16	14	14	17.7	15.1-19.4	1.41	12.5-25.0	18.0
11	18	15	15	14.5	13.2-15.8	1.42	10.2-20.5	15.3
11	19	15	15	21.2	19.9-22.6	1.28	16.6-27.1	21.8
11	20	15	15	22.1	20.3-24.2	1.40	15.9-31.0	23.5
11	21	15	15	11.1	16.7-19.6	1.36	13.3-24.6	18.9
11	22	15	15	19.7	18.1-21.3	1.38	14.3-27.1	20.4
11	23	15	15	19.7	18.4-21.4	1.31	15.0-25.9	20.5
11	24	15	15	25.5	23.7-27.2	1.29	19.6-33.0	26.3
12	25	14	14	21.1	19.6-22.7	1.34	16.1-27.6	21.5
12	27	14	14	22.2	20.7-23.9	1.32	15.7-29.3	23.0
12	29	14	14	13.0	11.8-14.4	1.45	8.9-18.9	13.8
13	Nov 1	14	14	9.6	8.6-10.9	1.50	6.4-14.4	10.3
13	4	14	14	15.5	14.3-16.8	1.34	11.5-20.8	16.1
13	6	15	15	15.7	14.5-17.1	1.38	11.4-21.0	16.5
14	8	15	15	15.3	14.4-16.3	1.28	11.7-19.7	15.8
14	11	15	15	18.5	17.3-19.7	1.28	14.4-23.6	19.0
14	13	15	15	17.3	16.3-18.8	1.26	13.7-21.9	17.8
15	15	15	15	17.6	16.6-18.8	1.27	13.9-22.3	18.1

ALL FINDINGS GROUP Y

WEEK	Number	Date	No. of MEN	MEAN ⁵	DISPERSION		MEAN ¹
					68% RANGE of MEAN ⁵	68% RANGE	
8 Sept 27	14	14	20.1	18.8-21.6	1.30	15.5-26.2	20.8
8 Oct 2	14	14	20.4	18.5-22.5	1.45	14.0-29.6	21.5
9 4	14	14	20.7	19.3-22.0	1.28	16.2-26.4	21.2
9 9	14	14	19.1	17.9-20.3	1.28	14.9-24.3	19.5
10 11	14	14	20.4	19.3-21.6	1.23	16.6-25.2	20.5
10 16	13	13	19.2	17.5-21.2	1.41	13.7-23.1	20.3
11 18	13	13	14.7	13.9-16.0	1.38	10.7-20.2	15.4
11 19	14	14	20.1	18.9-21.3	1.25	16.1-25.1	21.6
11 20	14	14	22.5	21.1-24.0	1.27	17.7-28.6	23.1
11 21	14	14	19.1	17.8-20.4	1.30	14.7-24.7	19.6
11 22	14	14	20.3	18.9-21.7	1.30	15.6-26.3	20.8
11 23	14	14	20.8	19.5-22.2	1.27	16.4-26.4	21.3
11 24	14	14	24.3	22.9-25.7	1.25	19.5-30.2	24.2
12 25	14	14	22.0	20.5-23.0	1.24	17.7-27.4	22.5
12 27	13	13	24.8	23.1-26.8	1.31	19.0-32.5	25.7
12 29	13	13	13.5	12.5-14.5	1.30	10.4-17.5	13.8
13 Nov 1	14	14	10.1	9.1-11.0	1.42	7.1-14.3	10.6
13 4	14	14	9.9	9.1-10.8	1.37	7.2-13.7	10.6
13 6	13	13	5.7	4.9-6.5	1.63	3.5-9.2	6.3
14 8	14	14	7.5	6.9-8.2	1.40	5.4-10.6	7.9
14 11	14	14	13.0	12.2-14.0	1.30	10.1-16.7	13.4
14 13	14	14	14.0	12.9-14.5	1.25	11.0-17.1	14.0
15 15	14	14	12.4	11.7-13.2	1.26	9.8-15.7	12.7

MEAN⁵ PLASMA ATABRINE LEVELS OF MEN IN THE JUNGLE GROUP - REARRANGED FOR STUDY OF DREAM AND RECOVERY

MEAN_G AND CHARACTERISTICS OF
PLASMA ATABRINE LEVELS
ON

1. GROUP OF MEN (COMPANY C) RECEIVING NO DRUG AFTER 11 WEEKS OF SUPPRESSIVE DOSE
2. GROUP OF MEN (COMPANY B) RECEIVING THERAPEUTIC DOSE AFTER 11 WEEKS OF SUPPRESSIVE DOSE

Drug Discontinued

Company C Section 1

WEEK	Number	Date	NO. of MEN	MEAN _G	68% RANGE of MEAN _G	DISPERSION		MEAN _A
						2 σ	68% RANGE	
12	Nov	13	32	12.8	12.8-13.4	1.34	9.5-17.1	13.5
12		15	39	9.6	9.0-10.1	1.42	6.7-13.5	10.2
12		17	39	9.8	6.6-9.6	1.42	6.4-12.9	9.6
13		20	39	6.3	5.6-6.8	1.67	3.8-10.5	7.2

Therapeutic Dosage

Company B Section 1

WEEK	Number	Date	NO. of MEN	MEAN _G	68% RANGE of MEAN _G	DISPERSION		MEAN _A
						2 σ	68% RANGE	
12	Nov	18	42	45.9	44.0-48.0	1.33	34.6-61.0	47.9
13		19	41	45.0	43.1-47.1	1.33	33.8-59.8	47.0
13		20	39	42.0	40.1-44.0	1.33	30.3-56.0	46.6

Company C Section 2

12	Nov	13	44	17.0	16.3-17.3	1.33	12.8-22.5	17.6
12		15	45	12.8	12.1-13.5	1.41	9.1-16.0	13.5
12		17	43	11.8	11.3-12.4	1.34	8.9-15.8	12.3
13		20	44	10.5	10.0-11.0	1.39	7.6-14.6	11.1

Company B Section 2

12	Nov	18	40	47.0	43.1-48.6	1.25	37.7-58.5	48.1
13		19	41	49.7	47.8-51.5	1.27	39.0-63.2	51.1
13		20	41	48.4	46.7-50.1	1.26	38.6-60.8	49.6

APPENDIX C

SECTION V - Factors Related to Variability in Individual Plasma Levels.

1. Excretion and Degradation.

a. Discussion:

- (1) It became apparent early in the study that individual plasma atabrine levels may differ markedly from the group mean concentration, and that the levels attained were, to a large extent, characteristic of the individuals. Thus, certain men maintained high levels throughout the period of the study, whereas others had levels consistently below the group average. Consideration of the factors which regulate the plasma level led to several lines of inquiry which, it was hoped, might make clear the basic cause of these differences.
- (2) Atabrine entering the portal circulation during the initial absorption period is very rapidly removed from the blood stream. The final level which results from a given dosage schedule is a balance between the total rate of removal and the rate of entry of the drug. From a long-term point of view, the rate of entry following single doses is not significant provided the same total quantity is absorbed. No investigation of the completeness of absorption has been made but it is not believed that it could be responsible for the large differences found in equilibrium plasma levels. Previous investigations reported by Shannon on the fecal excretion of the drug in animals tends to bear out this belief. The second factor which determines the balance, the rate of removal of the drug, is the resultant of several individual rates of removal by different avenues. Among these are (a) extraction of atabrine from the blood stream into the tissues where it accumulates in high concentrations, (b) degradation or destruction, and (c) urinary excretion. The plasma level of atabrine measured 24 or 48 hours after the last dose of the drug is determined by, and reflects the tissue concentration with which it is in equilibrium. Constancy of the plasma atabrine level over a period of time with a uniform rate of intake, therefore, indicates that the tissue concentration has become stabilized and that the rate of removal (sum of items b and c above) has become equal to the rate of intake. Thus, an unusually high plasma level

might result from (a) decreased rate of degradation; (b) a decreased rate of excretion or (c) a combination of both. Atabrine destruction is believed to occur largely in the liver. The possibility arises, therefore, that some impairment of liver function might be present in men with high plasma levels. Moreover, measurement of excretion rates should indicate whether variation in rate of loss into the urine contributes significantly to the differences in plasma level.

b. Procedure: Liver function tests were applied as follows to men whose plasma atabrine levels had remained with some consistency in either the upper or lower ranges of their group: bromsulfalein excretion, prothrombin time, plasma fibrin concentration, and icterus index. Brief descriptions follow:

- (1) Bromsulfalein excretion. 5 mg/kilo were injected intravenously in 1 minute. Blood samples were taken 6, 15 and 30 minutes after the start of injection. Heparin was used as an anticoagulant. 0.5 ml of plasma were diluted with 2 ml of an M/15 phosphate buffer (6 parts of Na_2HPO_4 to 4 parts of KH_2PO_4) and read in a spectrophotometer at a wave length of 590 m μ . After the addition of 0.06 ml of 2N NaOH a second reading was made at the same wavelength. A concentration of 10 mg bromsulfalein/100 ml plasma was considered 100%. The half excretion time was estimated graphically, using semilogarithmic paper.
- (2) Prothrombin time. The procedure of Page (J. La. & Clin. Med. Page 26: 1366, '41), using viper venom as a thromboplastic agent was employed. Estimations were made at several dilutions of the plasma in saline in the hope of revealing any differences which might have been concealed as a result of the undiluted prothrombin time falling on the asymptotic portion of the time-dilution curve.
- (3) Icterus index. The acetone procedure of Newburgh (J. Lab. Clin. Med., 22: 1192 (1937)) was used.
- (4) Plasma fibrin. Plasma was collected as for prothrombin time (1 ml of M/10 potassium oxalate for each 9 ml of blood). 1 ml plasma was diluted with 4 ml of saline, 1 ml of 0.025 molar CaCl_2 was added, the fluids mixed and allowed to stand 2 hours at room temperature. The clot was separated and washed in the same tube by centrifugation, once with 10 ml saline and twice with the same amounts of distilled water. The fibrin was finally transferred to a flask and the nitrogen determined by micro-Kjeldahl. The factor 6.25 was used to convert to protein.

c. Results.

- (1) The results of the liver function tests are to be found in Table (17)* and are plotted in Charts (34 and 35). There is no necessity for detailed discussion. Each of the tests indicates that there is no correlation between plasma level of atabrine and the hepatic functions measured. The tests all yielded results within the normal ranges. Owing to the insensitivity of liver function tests these findings are not necessarily conclusive in eliminating liver impairment as one factor responsible for the high plasma levels. There is the possibility that normal efficiency with respect to the functions measured may have existed along with a reduced ability to degrade atabrine. One may however, on the basis of these findings, eliminate gross differences in liver efficiency as a significant factor.
- (2) Similarly, the results of the urine studies (Table 18 and Chart 36) failed to show differences in urinary excretion that would explain the differences in plasma concentrations. Both of the men with high plasma levels excreted more atabrine in the urine than did the low men. The possibility that the high plasma levels are elevated because of decreased loss in the urine is thus eliminated.

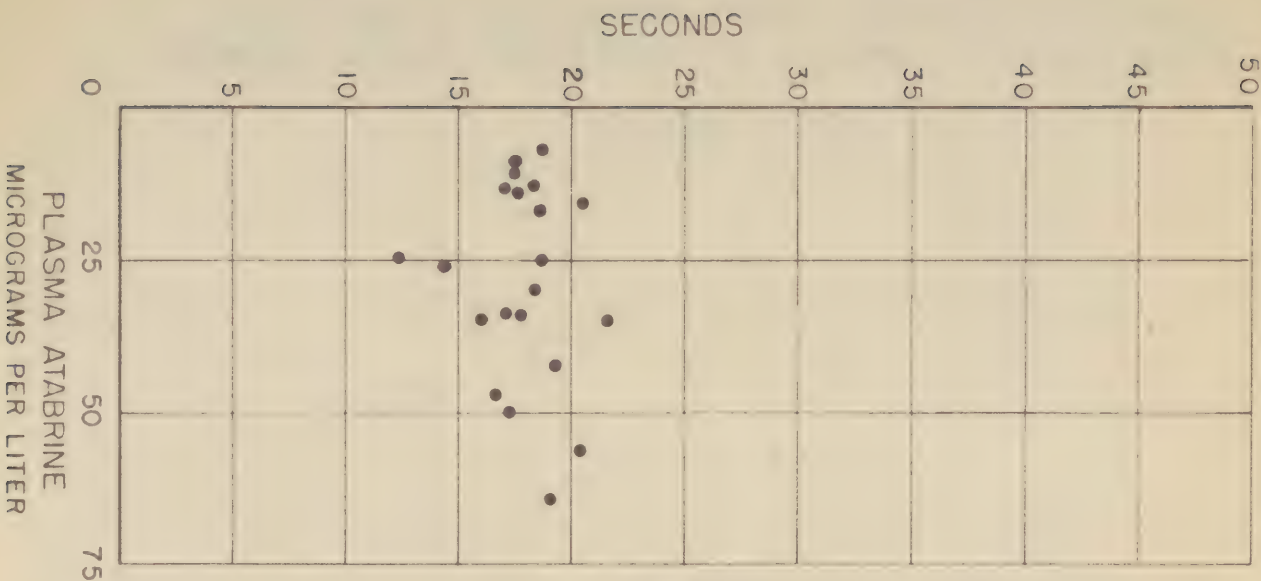
2. Variation in Protein Binding.

a. Discussion: There remains for consideration another independent factor which may explain variations in individual plasma levels, namely, differences in the amount of bound or non-active atabrine in the plasma with little or no variation in the free or active fraction of the drug. Since the latter constitutes only 10 to 20 per cent of the total, it is evident that the overall level is largely determined by the amount of bound atabrine. Both the concentration and total amount of atabrine in plasma are very small compared to that stored in the rest of the body. One may anticipate that after the transient changes in concentration incident to movement of the atabrine into the tissues, the free atabrine concentration found in the plasma will be determined by the resultant balance of the concentrations in the various tissues, determined in turn by the respective partition coefficients between these tissues and the plasma water and extra-cellular fluid. From this it follows that whereas the concentration of free atabrine will be related to the net tissue concentration, the whole plasma concentration will be only an indirect measure of tissue

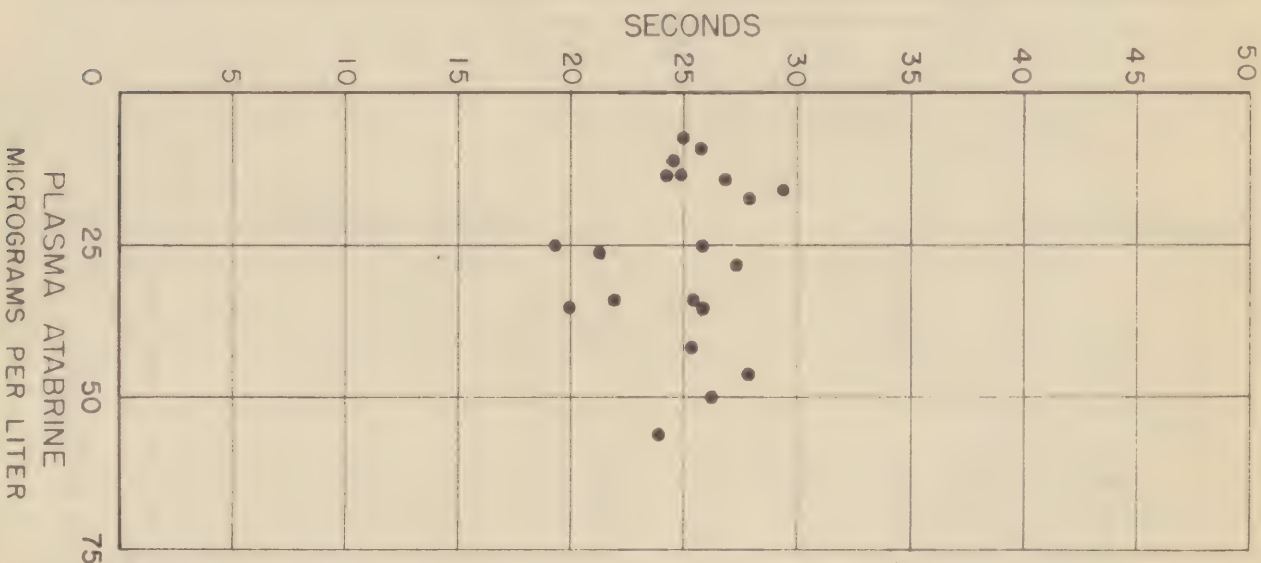
* Tables will be found at end of section.

RELATIONSHIP OF PLASMA PROTHROMBIN TIME TO PLASMA ATABRINE LEVEL

PROTHROMBIN TIME
UNDILUTED



PROTHROMBIN TIME
DILUTED 2 TIMES



PROTHROMBIN TIME
DILUTED 4 TIMES

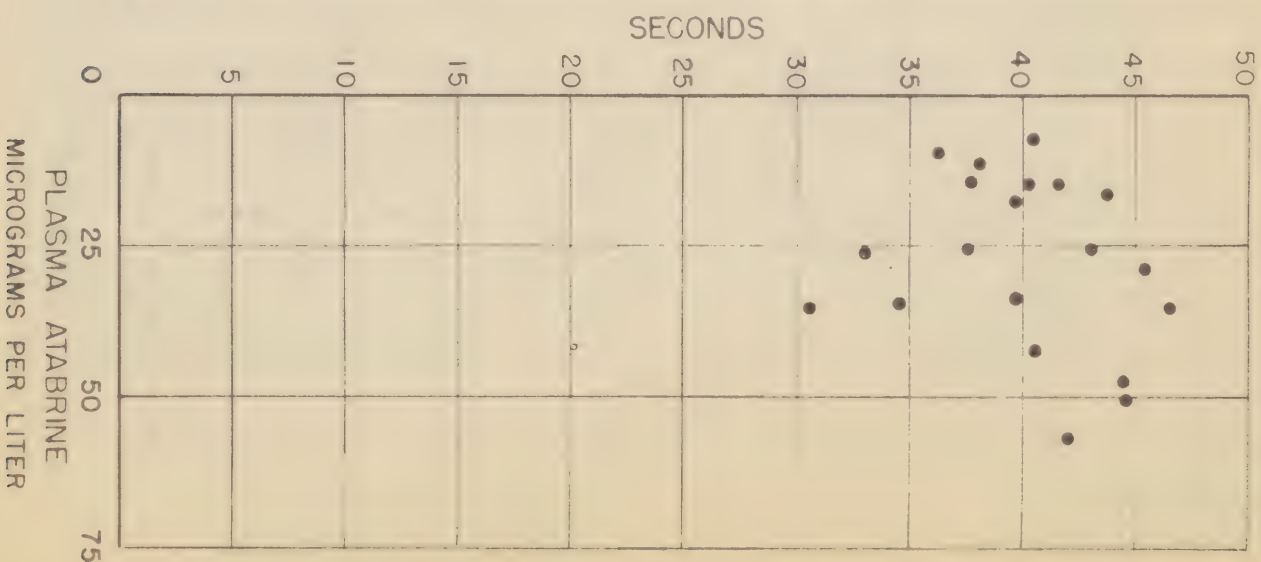
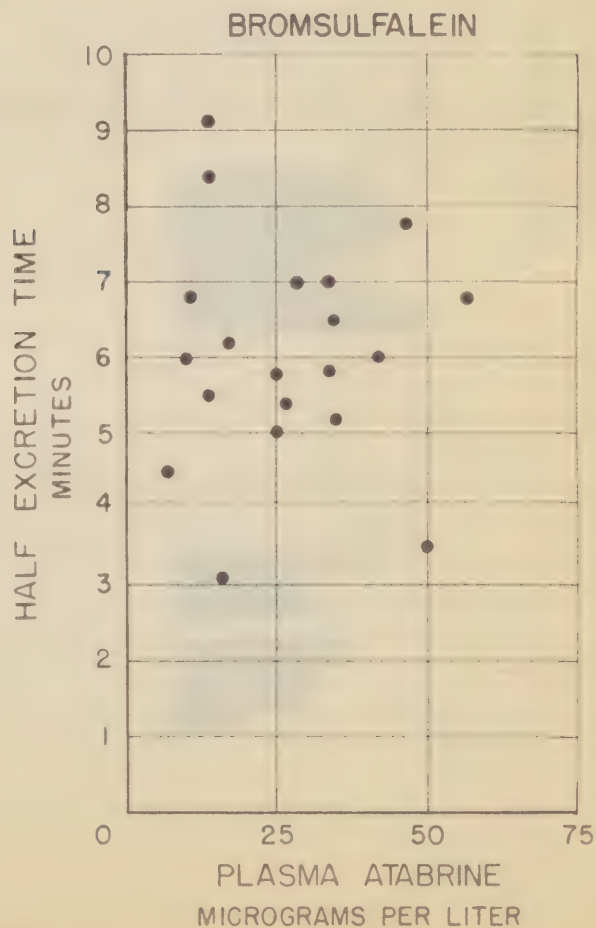
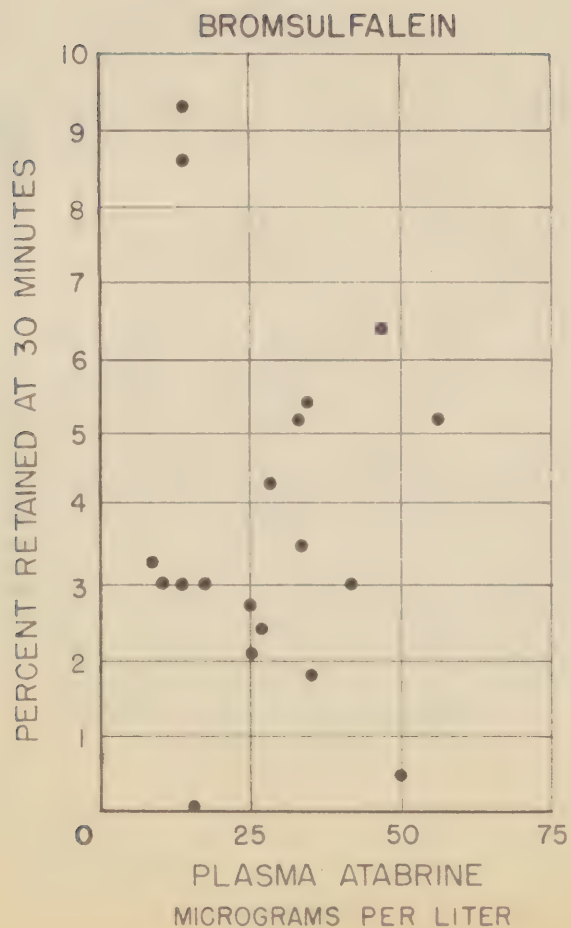
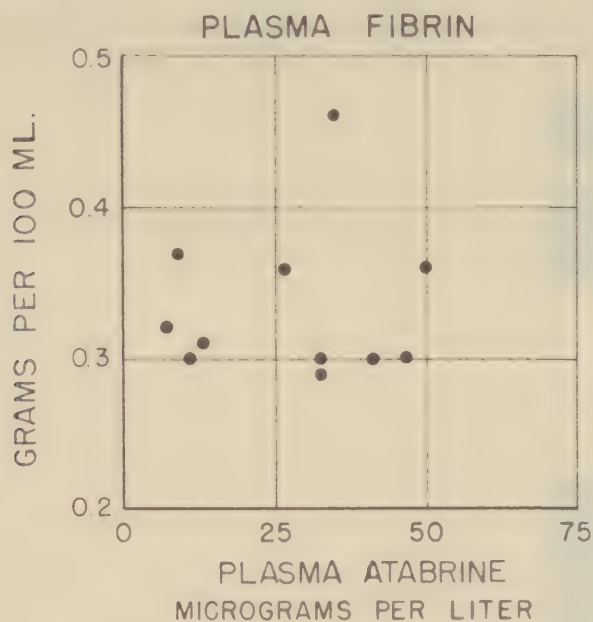


CHART — 35

RELATIONSHIP OF PLASMA FIBRIN CONCENTRATION AND
BROMSULFALEIN EXCRETION TO PLASMA ATABRINE LEVEL



24 HOUR EXCRETION OF ATABRINE IN RELATION TO PLASMA ATABRINE LEVEL

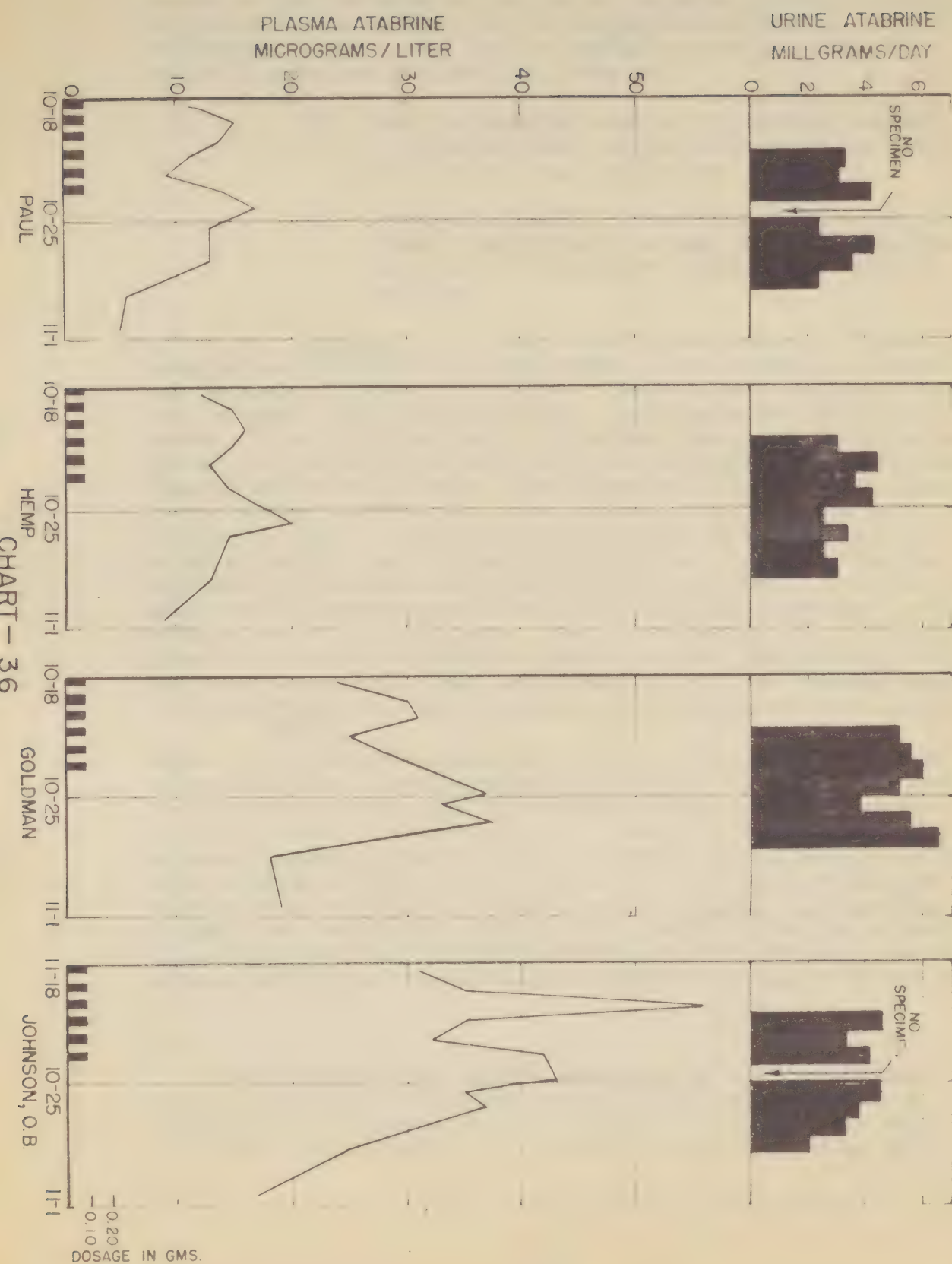


CHART - 36

concentration, reliable only so far as the extent of protein binding in plasma is predictable. It was not feasible to examine this possibility directly, but from incidentally accumulated data the following questions are partially answered:

- (1) Does the atabrine level of the cellular elements of the blood bear a linear relation to the plasma level in both high and low men?
- (2) Is the relationship between free atabrine and whole plasma atabrine more, or less, constant than the relationship between free atabrine and cellular atabrine?

b. Procedure: Cellular atabrine measurements. In most cases atabrine was estimated from measurements of whole blood concentration, and per cent of plasma in the blood. These values were then corrected to a standard (7500) leukocyte count. An example will illustrate the method. When the whole blood atabrine was found to be 96 micrograms/L, plasma from the same specimen gave 28 micrograms/L. Since the plasma constituted 58% of the whole blood, there was $0.58 \times 28 = 16$ micrograms in the plasma, and $96 - 16 = 80$ micrograms atabrine in the cells from 1 liter of blood. If we assume that atabrine is present in the erythrocytes in twice the concentration it is in plasma* then the erythrocyte concentration is $2 \times 28 = 56$ micrograms/L. The whole blood contained 42% erythrocytes, whence $0.42 \times 56 = 24$ micrograms in the erythrocytes of the cell phase. Thus, there was $80 - 24 = 56$ micrograms in the white cells from 1 liter of blood. The leukocyte count was found to be 8600. Thus for a count of 7500, the atabrine would have been $\frac{7500}{8600} \times 56 = 49$ micrograms in the leukocytes from 1 liter of

blood. This, plus the estimated content of the erythrocytes of 24 micrograms which is now returned to the total, gives the corrected cellular atabrine value of 73 micrograms which corresponds to the atabrine content of the cells from 1 liter of blood corrected to a standard leukocyte count of 7500. The symbol Ac' is used to designate this quantity in Table 20. The assumed value for the erythrocyte concentration is uncritical in fixing the final value; if it has been assumed that there was no atabrine in the erythrocytes the corrected value would have been 70 micrograms instead of 73, while for twice the assumed erythrocyte concentration the corrected value would have been

* Although this is not a valid assumption, it is acceptable here owing to the fact that the absolute value employed makes little difference in the correction (see further discussion in the text). The factor of 2 probably does represent a reasonable median value.

76. This arises from the fact that the chief contribution to the cellular atabrine is that portion contained in the white cells. In a few experiments effort was made to analyze the several phases in as pure a form as possible. The leukocyte phase was isolated reasonably free from red cells by the following technic: The whole blood was centrifuged for 10 minutes at 1000 rpm, the supernatant plasma was aspirated into a clean dry syringe down to the buffy layer. The buffy layer was then aspirated into another syringe, transferred to a Kahn tube, diluted with plasma to a volume of approximately 3 ml, mixed and recentrifuged at 800 to 1000 rpm for 1 minute. The supernatant of the last centrifugation gave a suspension of white cells in plasma two to three times more concentrated than in the original blood. The suspension was relatively free from erythrocytes. In our hands this procedure for isolation of the leukocytes frequently led to a serious error, owing, perhaps to a preferential sorting or selection of the leukocytes according to size or type during the double centrifugation. It offered no advantage over an estimation of the cellular atabrine content based upon the levels in plasma, whole blood, and red cells as outlined above. Centrifugation of the packed red cells for 1/2 to 1 hour at 2000 rpm yielded a phase in which plasma was present to only a small percent and white cell counts were regularly below $50/\text{mm}^3$. Comparison of a series of whole blood values reached by direct analysis with values secured by combination of the results on the separate phases is made in Table 19.

c. Results:

(1) Non-proportionality of cellular and plasma atabrine values.

- (a) The findings are collected in Table 20. In Chart 37 the corrected values for cellular atabrine and the ratios of plasma to cellular levels are plotted against the corresponding plasma atabrine concentrations. As previously mentioned, the men studied were selected from the extreme ranges of their respective groups. Chart 37 shows that there is a fairly definite correlation between the two measures of atabrine concentration, but not a directly proportional relationship such as would result if the partition coefficient which governs the distribution was the same in all men. The extent of the departures from a direct relation has been indicated by plotting the ratio of plasma atabrine to cellular atabrine against plasma atabrine. The ratio is not constant but varies with plasma concentration from $\frac{1}{10}$ for low plasma levels to $\frac{1}{3}$ for high levels; that is to say, for a four-fold

PROPORTIONALITY BETWEEN PLASMA ATABRINE AND CELLULAR ATABRINE

0.6 GM. GROUP

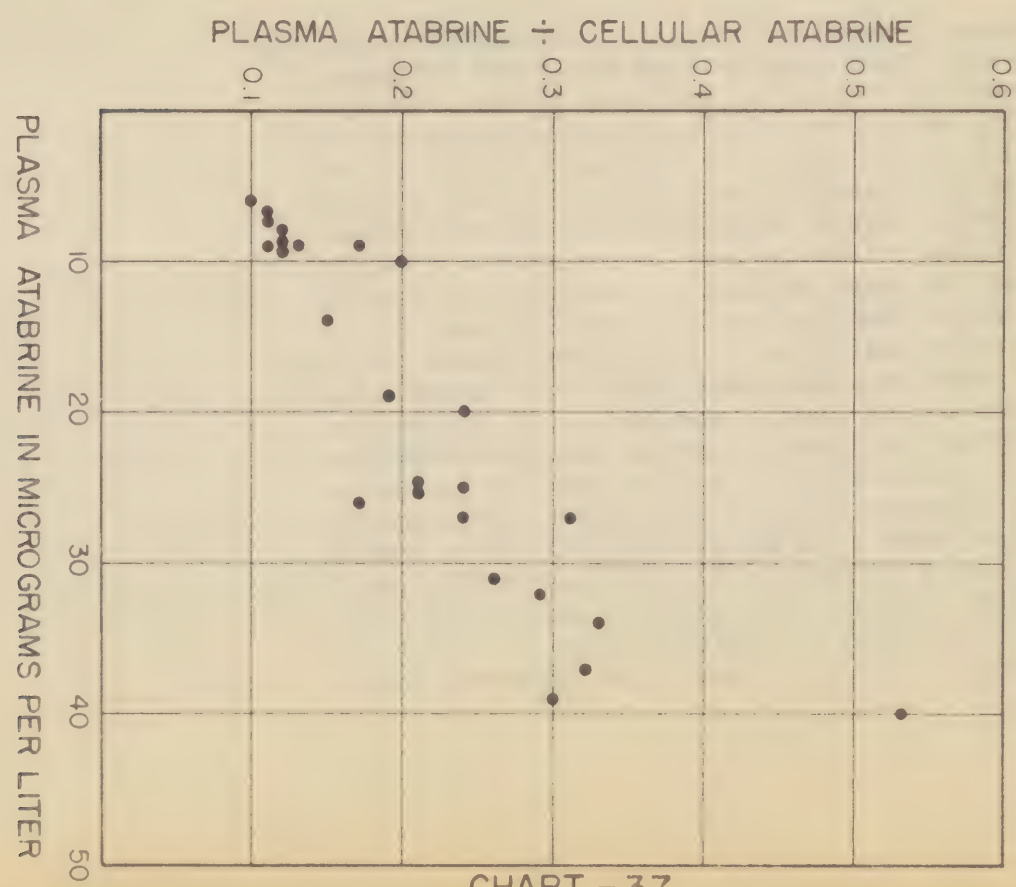
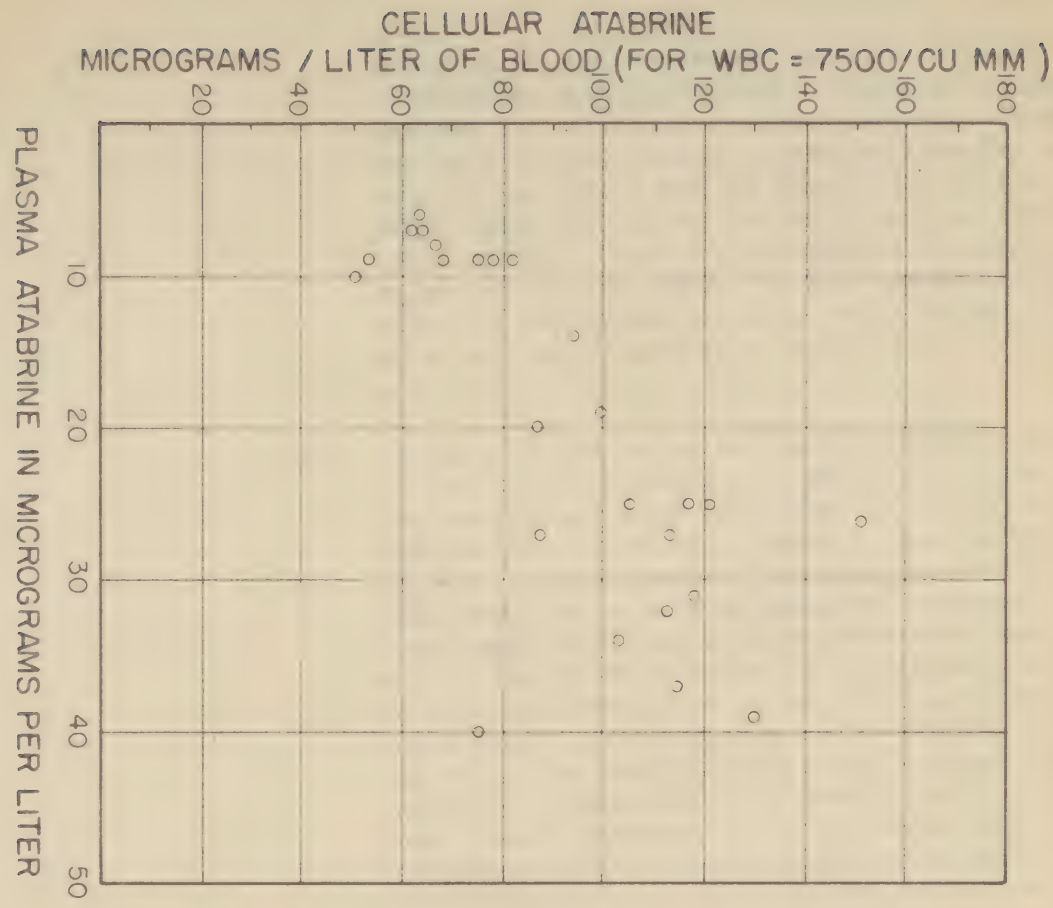


CHART - 37

CHART - 37

range in plasma concentration the cellular concentration is only doubled. The same general pattern is exhibited by the relation between erythrocyte and plasma concentrations for a smaller series of men in Chart 38. On the other hand, leukocyte and erythrocyte concentrations appear (Chart 39) to bear a constant relation to each other for all concentrations, which is consistent with an equilibrium governed by a uniform distribution coefficient for all men.

- (b) Failure of the cellular atabrine concentration to increase in direct proportion to plasma concentration might be explained on the grounds of saturation of the cell phase at low plasma levels, making further increase impossible. This explanation was eliminated, however, by an experiment in which erythrocytes were equilibrated with a solution of atabrine in Ringer's solution of such concentration as to yield a normal erythrocyte concentration and a similar solution in which the atabrine was about 9 times as concentrated. Approximately equal distributions were found, however, at both levels (see Table 21) indicating that the original erythrocyte concentrations were far below saturation levels.
- (2) Lesser dispersion of cellular atabrine values. The geometric means and the standard deviations of two separate sets of parallel cellular and plasma atabrine levels are given in Table 22 for the two groups of men on dosage schedules of 0.4 gm/wk and 0.6 gm/wk respectively. A comparison of these statistical values reveals two significant facts: first, the standard deviations of the values for cellular atabrine levels are far smaller than for the plasma values (and incidentally are not much higher than the standard deviations of the plasma values of the large group from which these extremes were drawn) and second, while the mean plasma values for these extreme cases bear no relationship to the dosage received, the mean values of the cellular atabrine levels differ roughly in direct proportion to the dosage rates (0.4 and 0.6 gm/wk, respectively). These differences are illustrated in a somewhat different manner in Chart 40. Here are plotted on the same time scale, plasma and cellular atabrine values for eight men, four from the 0.4 gm/wk group

PROPORTIONALITY BETWEEN PLASMA ATABRINE AND RED CELL ATABRINE

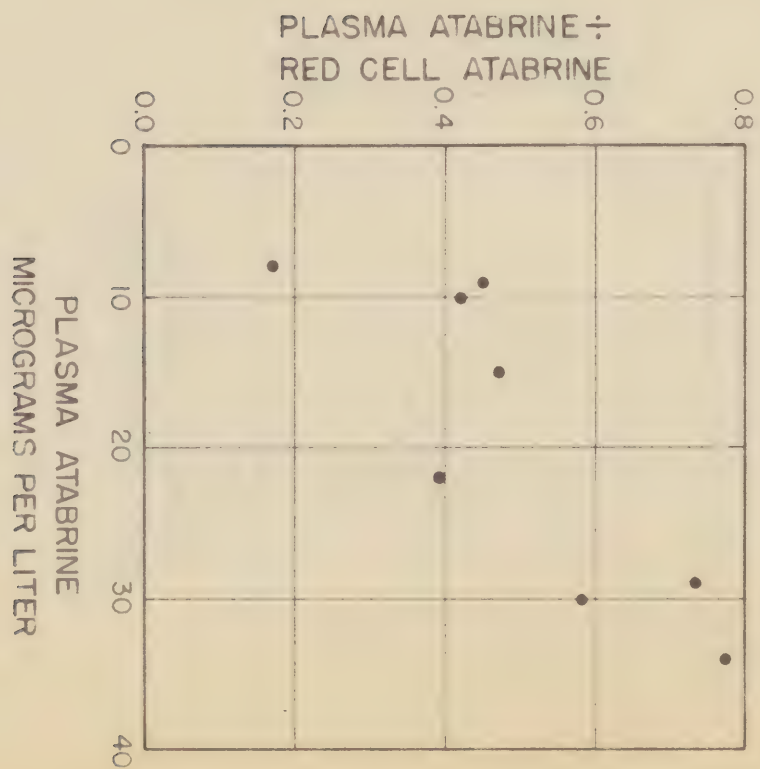
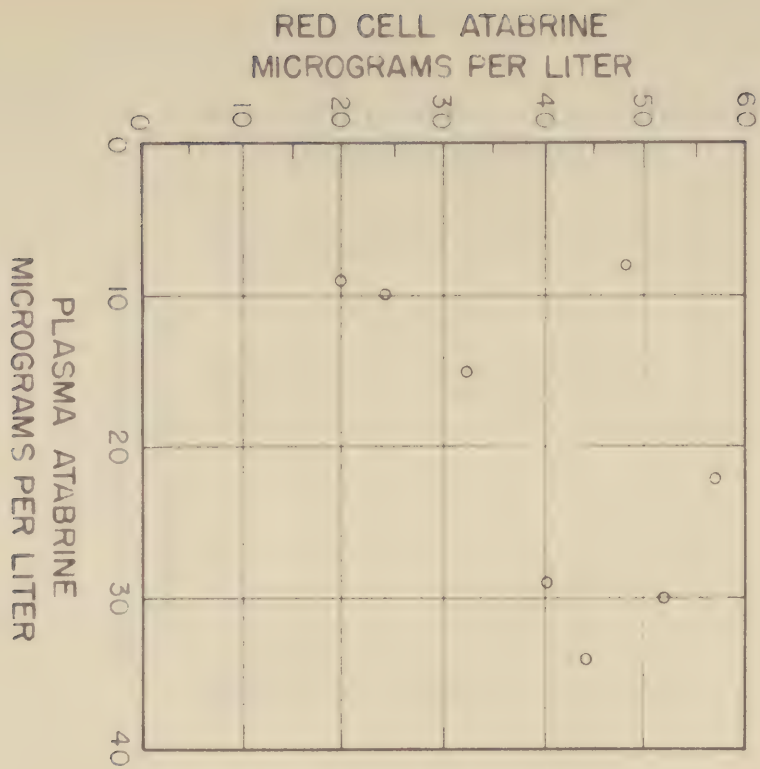


CHART - 38

PROPORTIONALITY BETWEEN WHITE CELL ATABRINE AND RED CELL ATABRINE

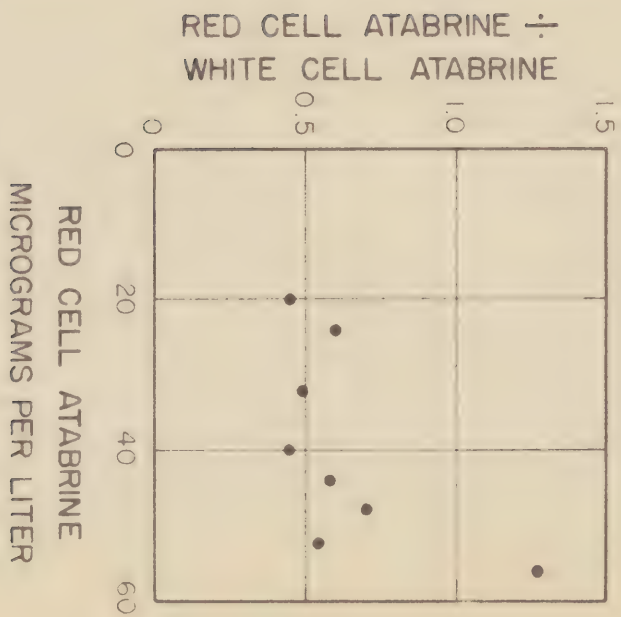
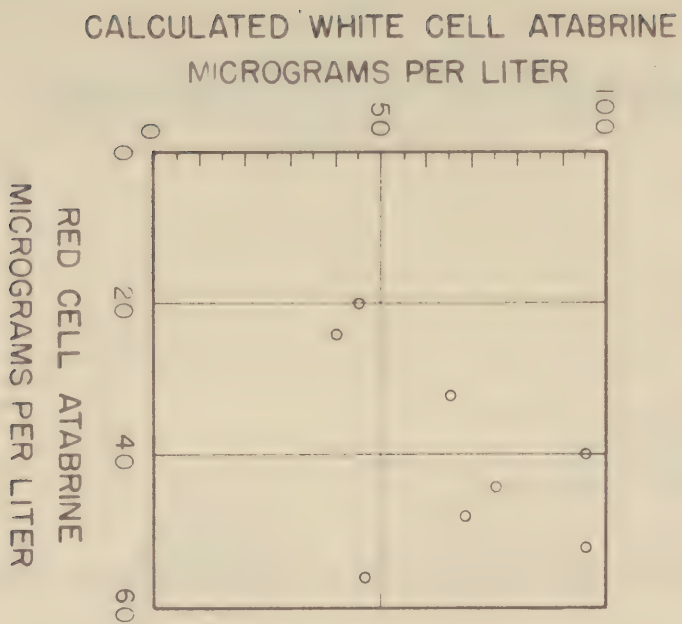


TABLE 21

ATABRINE PARTITION
Between Phosphate Ringer's and Erythrocytes
37.5°, pH 7.25

Name	Plasma Atabrine Micrograms/L	Final Ratio <u>Erythrocyte Phase</u> Supernatant	
		Supernatant Conc. =	
		Approx. 2.5 Micrograms/L	Approx. 23 Micrograms/L
Weiss	14	10	12
Ostrowitz	17	10	11
Canada	5	8	(11)
Vandertie	5	10	13

TABLE 21

TABLE 22

COMPARISON OF MEANS AND RELATIVE DISPERSIONS
OF PLASMA ATABRINE LEVELS AND CELLULAR ATABRINE LEVELS

	Dosage 0.4 gm/week (B-1 & C-1)		Dosage 0.6 mg/week (B-2 & C-2)	
	Plasma Atabrine Ap	Cellular Atabrine Ac'	Plasma Atabrine Ap	Cellular Atabrine Ac'
Date	10-29, 30		10-29, 30	
Number of Men	11	11	13	13
Mean _G	17.6	65.7	16.2	84.5
σ_g	1.96	1.31	1.95	1.40
Date	11-8, 9		11-5, 6	
Number of Men	11	11	12	12
Mean _G	16.6	68.2	20.3	95.1
σ_g	1.79	1.33	1.79	1.35

Ap -micrograms per liter

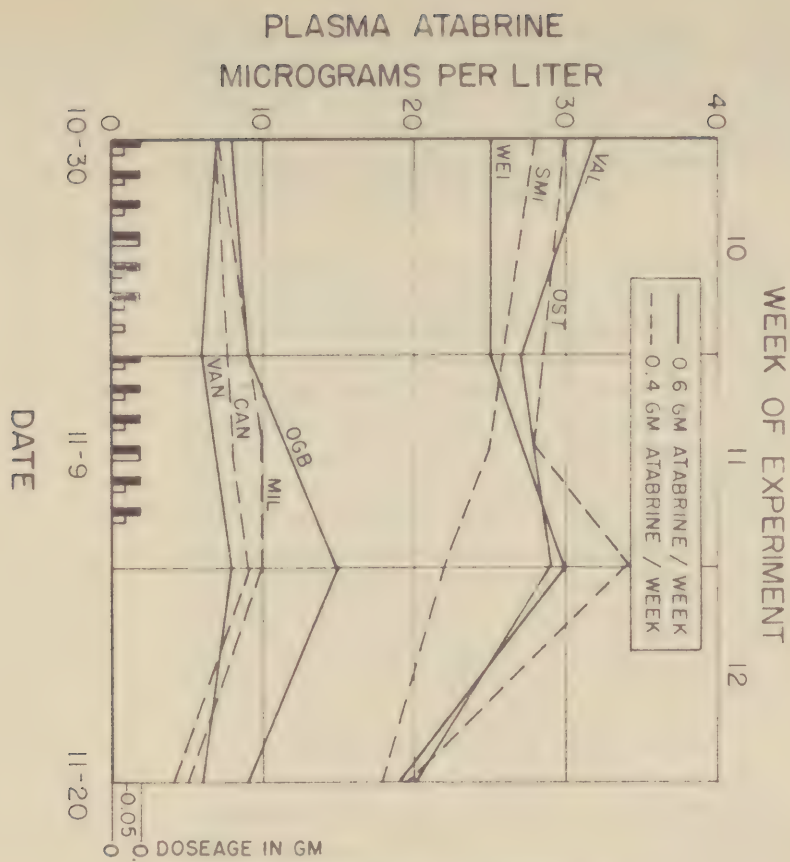
Ac'-micrograms in the cells from 1 liter of blood for a white count of 7500

TABLE 22

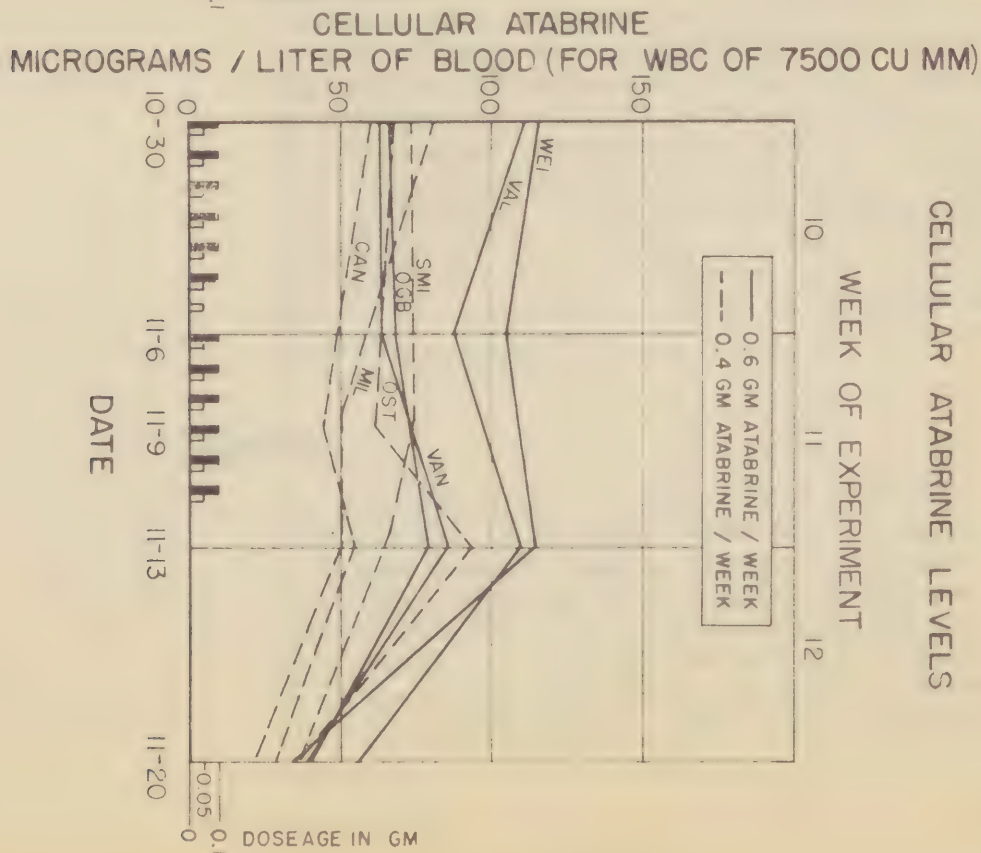
COMPARISON OF PLASMA AND CELLULAR ATABRINE LEVELS

IN EIGHT SUBJECTS

PLASMA ATABRINE LEVELS



CELLULAR ATABRINE LEVELS



and four from the 0.6 gm/wk group. It can be seen that in the case of the cellular atabrine levels, in contrast to plasma concentrations, definite groupings of the men according to dosage is achieved. Moreover, a marked reduction in the difference between the high and the low men is apparent.

- (3) Equilibration experiment. Limitations of time made it impossible to carry the investigation to the logical point of actually estimating the concentration of free atabrine in the plasma. This probably can be done with sufficient accuracy for the purpose by the procedure described by Brodie and Shannon. It involves equilibration of erythrocytes with a sufficient series of atabrine-containing, protein-free, media to permit determination of the partition coefficient of that particular lot of erythrocytes. Some of this lot of erythrocytes are then equilibrated with the plasma in question, separated and analyzed. Then, knowing the partition coefficient of the erythrocytes, the free atabrine concentration in plasma can be estimated. A similar but incomplete experiment was carried out during this study in which red cells from 2 high and 2 low men were equilibrated with 2 different concentrations of atabrine in a buffered Ringer's solution. At the end of one hour at 37.5°C the cells were separated by centrifugation and the two phases from each equilibration were analyzed. The results are assembled in Table 21. There was insufficient material to permit an adequate analysis of the original concentration in the erythrocytes so that only very limited information can be drawn from this experiment. It is apparent, however, that (1) saturation was not reached, (2) the partition coefficients are substantially the same in the different cells. If the latter is true, we are then justified in giving even more weight to the greater uniformity of cellular atabrine.

d. Summary:

- (1) The suggestion is very strong that variation in protein binding is the main cause for the large differences in plasma levels found in the groups studied. This conclusion rests on three points arising out of the present studies: (a) The concentration of atabrine in the cellular phase of the blood does not vary among individuals in direct proportion to the plasma atabrine concentration. This lack of proportionality

between the two does not, in the case of a high plasma level, result from saturation of the cells with atabrine. It does, however, indicate that variation in one or more partition coefficients (plasma water to plasma protein, or plasma water to cells) does occur. (b) The concentrations of atabrine in the cellular elements of the blood show not only less relative dispersion than the plasma atabrine concentrations but are, in the case of individuals with extremely low or high plasma levels, more closely related to previous dosage than the plasma values. This point plus (a) tends to incriminate protein binding as a factor more variable than the "plasma water to cells" partition coefficient, since, until disproved, we must assume that men who have the same dosage history will have the same tissue concentration of atabrine. (c) One incomplete experiment suggested that partition between free atabrine and erythrocytes was reasonably uniform in 2 high and 2 low men, and independent suggestion that cellular atabrine may in many instances be a more reliable guide to the concentration of free atabrine in plasma than is the whole plasma concentration.

- (2) The information presented here is inconclusive as to the probable constancy or uncertainty of the free atabrine to protein partition. Sufficient doubt, however, is thrown on its previously assumed constancy to warrant a thorough investigation of this variation in an adequate sample of normal men. In this way it may be possible to evaluate more completely the limitations of the level of atabrine in whole plasma as an index of the levels of atabrine in other tissues - whether in those of a parasite or of the host.

TABLE 17

RESULTS OF SEVERAL INDICES OF LIVER FUNCTION
ON SUBJECTS WITH DIFFERENT LEVELS OF
PLASMA ATABRINE

NAME	DATE	GROUP	Days on Therapy	Grams Atabrine Taken	Plasma Atabrine Micrograms/Liter*	Prothrombin Time Seconds At Dilution			Bromsulfalein		Fibrin gm/100 ml.	Icterus Index
						0	$\frac{1}{2}$	$\frac{1}{4}$	Per-cent Retained at 30 min.	Half Excretion Time Minutes		
Canada	10-14	Co. C, S-1	48	2.7	7	18.8	25.0	40.6	1.2	4.5	0.32	5
Leskowsky	10-18	Co. B, S-2	53	4.5	9	17.4	25.8	36.1	3.3	6.0	0.37	5
Vandertie	10-12	Co. C, S-2	46	3.9	11	17.4	24.5	39.0	3.0	6.7	0.30	5
Pelczar	10-13	Co. B, S-1	48	2.7	13	18.1	24.9	37.9	3.0	5.5	0.31	5
Paul	9-18	A	41	4.1	14	17.4	26.7	40.1	9.4	9.2	-	-
Cox	9-29	A	52	5.0	14	17.2	24.2	41.5	8.7	8.4	-	-
Hemp	9-30	B	53	5.1	16	20.4	29.2	43.7	0.1	3.2	-	-
Littleton	9-29	A	52	5.0	17	18.6	27.8	39.6	3.0	6.2	-	-
Harry	9-29	A	52	5.0	25	12.4	19.2	37.5	2.1	5.0	-	-
Pachucki	9-30	B	53	5.1	25	18.6	25.9	43.0	2.8	5.8	-	-
O'Gara	10-12	Co. C, S-2	46	3.9	26	14.4	21.1	32.7	2.4	5.4	0.36	5
Perry	9-30	B	53	5.1	28	18.5	27.2	45.8	4.4	7.0	-	-
Bellesky	10-13	Co. B, S-1	48	2.7	34	17.8	22.0	34.5	5.2	7.0	0.29	5
Krawiecki	10-18	Co. B, S-2	53	4.5	34	17.2	25.4	39.6	3.5	5.9	0.30	5
Sink	9-18	A	41	4.1	35	21.5	25.6	46.8	5.3	6.6	-	-
Berka	10-12	Co. C, S-2	46	3.9	35	16.6	20.0	30.4	1.9	5.3	0.46	5
Smith	10-14	Co. C, S-1	48	2.7	42	19.3	25.3	40.6	3.0	6.0	0.29	5
Gordon	10-13	Co. B, S-1	48	2.7	47	16.7	27.8	44.7	6.3	7.7	0.29	5
Allison	10-18	Co. B, S-2	53	4.5	50	17.3	26.1	44.8	0.5	3.5	0.36	5
Johnson, O.B.	9-18	A	41	4.1	56	20.2	23.7	42.0	5.2	6.8	-	-

* Average of 4 values bracketing date noted (2H1's, H2's)

TABLE 17

TABLE 18

Excretion of Atabrine
in the Urine

Name	Plasma Atabrine Micrograms/L	Date	Urine Atabrine mg/L	Urine Vol ml./day	Urine Atabrine mg/day
Paul	11	10-21	2.0	1700	3.4
	9	10-22	1.66	1900	3.2
	14	10-23	2.46	1730	4.3
	13	10-25	1.18	2105	2.5
	-	10-26	1.26	3480	4.4
	13	10-27	2.48	1470	3.6
	-	10-28	0.90	2720	2.4
Average	12.0				3.4
Hemp	15	10-21	1.22	2450	3.0
	13	10-22	2.26	1960	4.4
	15	10-23	1.08	3390	3.7
	17	10-24	1.24	3410	4.2
	20	10-25	0.58	4400	2.5
	-	10-26	1.22	2810	3.4
	17	10-27	0.68	3790	2.6
	-	10-28	1.24	2370	2.9
Average	16.2				3.3
Golman	25	10-21	5.2	1000	5.2
	28	10-22	3.96	1420	5.6
	32	10-23	3.44	1720	5.9
	37	10-24	2.22	2360	5.2
	33	10-25	1.84	2080	3.8
	-	10-26	2.22	2530	5.6
	38	10-27	3.14	2110	6.6
Average	32.2				5.4
Johnson, O.B.	35	10-21	3.50	1320	4.6
	32	10-22	2.76	1200	3.3
	42	10-23	2.06	2060	4.2
	35	10-25	2.58	1780	4.6
	-	10-26	2.72	1450	3.9
	37	10-27	3.04	1120	3.4
	-	10-28	1.58	1300	2.1
Average	36.2				3.7

Line after 10-23 indicates discontinuance of drug.

TABLE 18

TABLE 19

Whole Blood
Atabrine
Micrograms/Liter

From Analysis of separate phases	From Analysis of whole blood
232	155
185	195
156	155
129	90
93	80
72	70
126	90
107	100

TABLE 19

TABLE 20
(Sheet 1)

PLASMA AND CELLULAR ATABRINE VALUES
ON SELECTED HIGH AND LOW MEN

GROUP * & SECTION	NAME	DATE	Cw**	Vc	Ap	Awb	Ac	Ac'	$\frac{Ap}{Ac'}$
B-2	Ihota	10-29	10.0	41.0	37	164	142	114	.32
B-2	Allison	"	7.5	42.0	40	98	75	75	.53
B-2	McMichael	"	8.6	36.0	31	152	132	118	.26
B-2	Krawiecki	"	9.0	39.0	27	148	132	113	.24
C-2	Valente	10-30	10.0	38.5	32	162	142	112	.29
C-2	Weiss	"	8.9	34.0	25	152	136	117	.21
C-2	Berka	"	6.5	30.5	19	100	87	99	.19
B-2	Elmore	10-29	7.8	43.0	7	70	66	62	.11
B-2	Farris	"	7.0	45.0	9	78	73	78	.12
B-2	Leskiowsky	"	9.8	38.0	9	70	64	53	.17
C-2	Ogborn	10-30	5.1	39.5	8	52	47	66	.12
C-2	Skrabis	"	10.0	39.5	9	102	97	75	.12
C-2	Vandertie	"	7.2	41.0	7	66	62	64	.11
B-1	Bellesky	10-29	10.5	41.0	30	98	80	64	.47
B-1	Gordon	"	8.4	40.5	39	122	99	92	.42
C-1	Ostrowitz	10-30	7.2	40.0	30	84	66	68	.44
C-1	Smith	"	8.6	42.0	28	96	80	73	.38
C-1	Nice	"	7.7	39.0	20	86	74	72	.28
B-1	Hildner	10-29	12.1	39.0	10	82	76	50	.20
B-1	Swigert	"	10.1	46.0	7	70	66	51	.14
B-1	Kirk	"	6.8	41.5	8	36	31	34	.24
C-1	Miller	10-30	4.05	39.0	7	50	46	81	.09
C-1	Woods	"	5.0	39.0	8	60	55	80	.10
C-1	Canada	"	5.9	38.0	7	52	48	60	.12

* Men in B-1 and C-1 received 0.4 gm Atabrine per week, those in B-2 and C-2 received 0.6 gm per week.

** Cw - Leukocyte count in whole blood, thousands/ cu. mm.

Vc - Red cell volume, per cent

Ap - Plasma Atabrine, micrograms/liter

Awb - Whole blood Atabrine, micrograms/liter

Ar - Red cell Atabrine, micrograms/liter

Ac - Cellular Atabrine, micrograms in the cells from 1 liter of blood

Ac' - Cellular Atabrine, micrograms in the cells from 1 liter of blood for a white count of 7500/cu. mm.

A₁' - Leukocyte Atabrine, micrograms in the leukocytes from 1 liter of blood for a count of 7500/cu. mm.

TABLE 20
(Sheet 2)

PLASMA AND CELLULAR ATABRINE VALUES
ON SELECTED HIGH AND LOW MEN

GROUP & SECTION	NAME	DATE	Cw	Vc	AP	Awb	Ac	Ac'	$\frac{Ap}{Ac'}$
B-2	Lhota	11-5	8.9	-	39	172	148	130	.30
B-2	Allison	"	5.4	-	34	102	82	103	.33
B-2	McMichael	"	8.4	-	26	182	166	151	.17
B-2	Krawiecki	"	8.7	-	25	152	137	121	.21
C-2	Valente	11-6	11.0	39.4	27	134	118	87	.31
C-2	Weiss	"	9.6	34.6	25	146	130	105	.24
C-2	Berka	"	8.8	34.8	20	110	97	85	.24
B-2	Elmore	11-5	6.4	-	14	90	82	94	.15
B-2	Leskowsky	"	9.9	-	10	70	64	50	.20
C-2	Ogborn	11-6	6.7	39.7	9	66	61	68	.13
C-2	Skrabis	"	7.0	42.0	9	82	77	82	.11
C-2	Vandertie	"	8.4	40.1	6	74	70	63	.10
B-1	Gordon	11-8	9.3	38.0	35	140	118	100	.35
B-1	Dark	"	8.5	39.9	24	158	144	129	.19
B-1	Fehrle	"	-	-	-	-	-	-	-
C-1	Ostrowitz	11-9	8.3	40.0	28	82	65	62	.45
C-1	Smith	"	11.0	40.2	25	112	97	74	.34
C-1	Niec	"	5.8	38.5	20	88	75	93	.22
B-1	Hildner	11-8	12.6	43.1	9	94	89	56	.16
B-1	Swigert	"	8.1	42.9	8	76	71	66	.12
B-1	Kirk	"	10.7	41.9	7	50	46	34	.21
C-1	Miller	11-9	9.0	41.4	10	62	58	50	.20
C-1	Woods	"	8.3	42.1	9	56	51	47	.19
B-1	Canada	"	8.4	38.9	8	54	49	44	.18

See sheet 1 for legend

TABLE 20
(Sheet 3)

PLASMA AND CELLULAR ATABRINE VALUES
ON SELECTED HIGH AND LOW MEN

GROUP & STATION	NAME	DATE	Cv	Vc	Ap	Awb	Ar	Ac	Ac'	$\frac{Ap}{Ac'}$	$\frac{Ap}{Ar}$	A_1'
C-2	Valente	11-13	9.7	41.0	29	155	40	133	110	.26	.73	94
C-2	Weiss	"	12.4	37.5	30	195	52	176	114	.26	.58	95
C-2	Ogborn	"	6.8	41.0	15	80	32	71	79	.19	.47	66
C-2	Vandertie	"	8.4	40.5	8	100	43	95	87	.09	.17	68
C-1	Ostrowitz	"	11.6	41.0	34	155	44	135	94	.36	.77	76
C-1	Smith	"	9.4	40.0	22	90	56	77	66	.33	.39	44
C-1	Miller	"	10.5	42.0	10	70	24	66	50	.20	.42	40
C-1	Canada	"	12.7	41.0	9	90	20	35	54	.17	.45	46
C-2	Valente	11-20	7.4	39.7	20	67	-	55	56	.36	-	-
C-2	Weiss	"	12.0	37.0	19	57	-	45	32	.58	-	-
C-2	Ogborn	"	8.6	39.9	9	50	-	45	40	.23	-	-
C-2	Vandertie	"	9.7	38.2	6	50	-	46	37	.16	-	-
C-1	Ostrowitz	"	8.9	33.0	20	57	-	44	39	.51	-	-
C-1	Smith	"	9.9	39.9	18	57	-	46	38	.47	-	-
C-1	Miller	"	11.0	42.0	5	33	-	30	22	.23	-	-
C-1	Canada	"	6.7	40.1	4	27	-	25	23	.14	-	-

See Sheet 1 for legend

TABLE 20

APPENDIX C

SECTION VI - Effects of Atabrine on Man.

1. Toxicity.

a. There were no unequivocal toxic reactions to atabrine in any of the 250 men on any of the suppressive dosage regimens. The health and well-being of all the men was excellent throughout. The A.R.T.C. group made an average gain in weight of 3 pounds during the experimental period and the group exposed to hot humid (jungle) climate manifested no disturbances that could be referred to the drug. In fact, the transient gastroenteric-tract disturbances commonly seen during the first days of exposure to heat were less marked in this experimental group than in others previously studied in the hot room. For the most part, the drug was given at meals; in instances however, it was taken between meals during periods of work in the sun.

b. All visits to the dispensary by men from both companies were carefully checked. In no instances could the complaints be attributed to atabrine nor was the frequency of visits by members of the experimental group greater than that of the men in the same units not taking the drug.

c. One case of probable toxic reaction from atabrine in therapeutic doses was encountered. The subject (Shackleford) had an epileptiform seizure on the 5th day of therapeutic dosage. Investigation revealed no evidence of previous seizures and there had been no disturbances while the subject was on suppressive therapy. On the day following the seizure the subject's plasma atabrine level was 108 micrograms, the highest attained by any of the group on the therapeutic regimen. Forty eight hours after the seizure the plasma level had fallen 67 micrograms, the administration of the drug having been continued throughout. No further incident occurred.

d. In view of the frequent reports of atabrine toxicity the failure to encounter such during the present study is of interest. This experimental group differed from men in combat theatres essentially in that the experimental group was not exposed to the psychological stresses of approaching combat. One may properly question therefore, if the drug, when administered in accordance with the schedule recommended, can be considered solely responsible for the reported complaints. Whether the drug plus emotional stress will account for the manifestations or whether symptoms due to stress and other factors are being attributed to the atabrine remains to be established.

2. Discoloration. The characteristic yellow discoloration from the drug began to be noticeable at the end of the first month and increased progressively thereafter. The intensity of color did not correlate with plasma levels.

APPENDIX D

IMPLICATIONS OF THIS STUDY AS APPLIED TO FUTURE INVESTIGATIONS

1. Field studies for determination of suppressive plasma level. It is generally agreed that one of the first points to be investigated in the field is the plasma level of atabrine which is necessary to suppress symptoms. For the preliminary organization and conduct of such an epidemiological investigation, the relationships established in the present study are directly applicable.

a. Selection of dosage regimens. The dosage required to develop any group mean equilibrium level that is desired can be obtained, as follows: Divide the value of the desired equilibrium level (in micrograms/L) by 209 to get the required daily dose in grams. Example: Desired level = 20 micrograms/L; daily dose required = $20/209 = 0.1$ gm/day. If it is desired to establish this level rapidly, administer double the daily maintenance dose, or 0.2 gm/day, for the first week.

b. Prediction of range and percentage distribution of individual equilibrium levels on a given regimen. The probable range of equilibrium levels attained by the individuals in a group on a selected regimen and the percentage of the group having concentrations below stated levels can be determined from Chart 41. Example: What percentage out of a group on a regimen of 0.1 gm/day will have plasma atabrine levels = 10 micrograms/L? Since the group mean equilibrium = $0.1 \times 209 = 21$ micrograms/L, the level in question = $\text{Mean}_G \times 0.5$. According to Chart 41, three (3) percent of the group will have plasma levels equal to or less than 10 micrograms/L. Similarly, 17 percent will develop plasma concentrations not greater than $\text{Mean}_G \times 0.7$ or 14 micrograms/L.

c. Determination of dosage regimen to produce equilibrium plasma levels above a desired minimum level in any given percentage of the population. In order to insure that a given percentage of the population will have plasma atabrine concentrations above a stated minimum level, the required dosage regimen is calculated as follows:

Divide the desired minimum by the ratio to the mean for the percentage in question as obtained from the log-probability line in Chart 41. The result is the required group mean plasma level. This value divided by 209 gives the necessary daily dose in grams. Example: What daily dosage is required to maintain plasma atabrine levels equal to or above 10 micrograms/L for all but one man in 100 (99%)? Referring to Chart 41, the minimum level which occurs 99 out of 100 times is found to be $0.42 \times \text{Mean}_G$; the group mean equilibrium level, therefore is $\frac{10}{0.42} = 23.8$ micrograms/L

and the required daily dose is $23.8/209 = 0.12$ gm/day. Similarly for all but one man in 1000 (99.9%) to be above 10 micrograms/L, the mean equilibrium level will be $\frac{10}{0.32} = 31.2$ micrograms/L and the required dosage is $31.2/209 = 0.16$ gm/day.

PREDICTED DISTRIBUTION OF PLASMA ATABRINE LEVELS

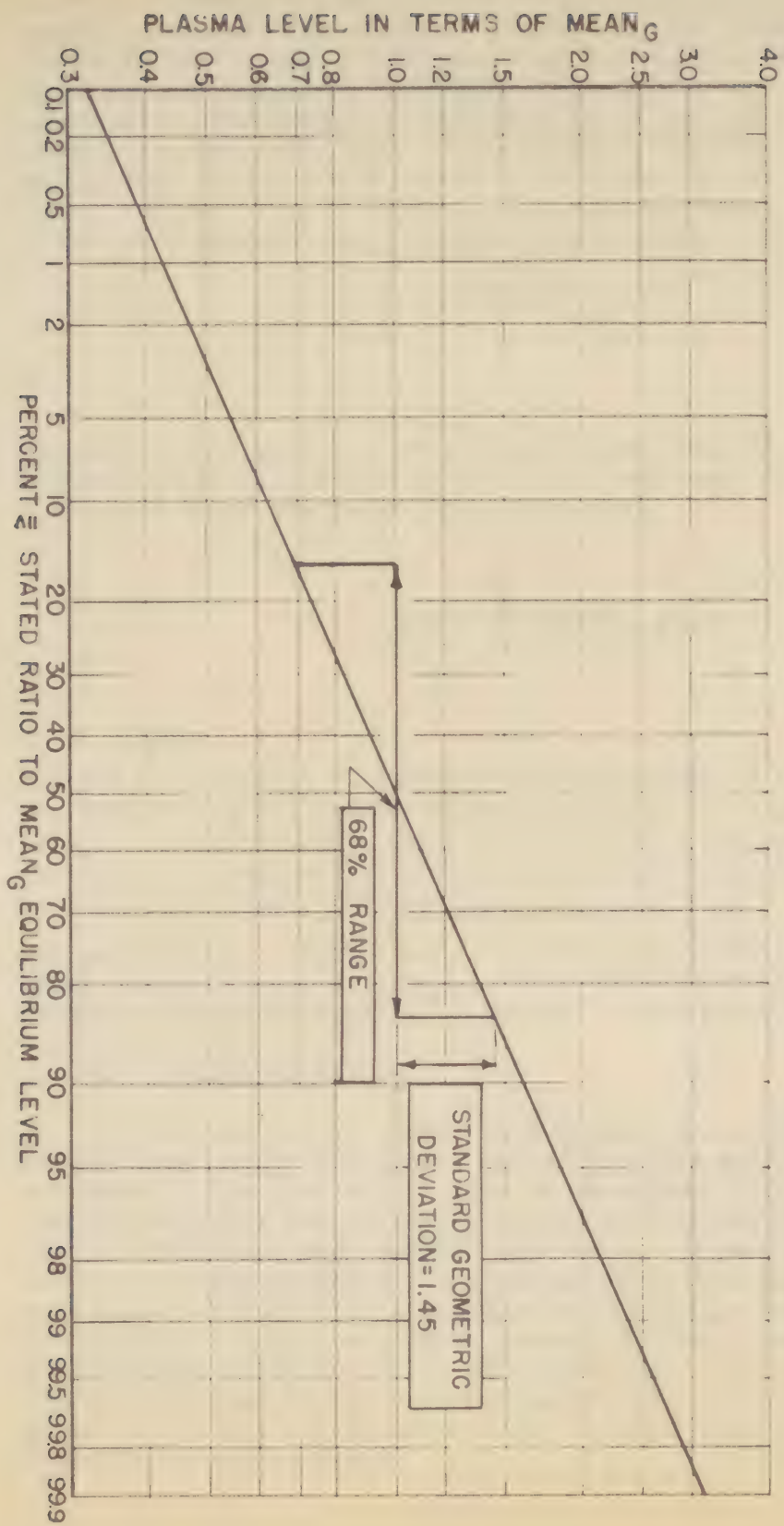


CHART-4I

d. Blood sampling for control. Any scheme for field study must be predicated upon the knowledge that the drug is being taken. In the absence of complete supervision of administration, some type of analytical control procedure is required. Since this will entail blood sampling and access to the troops, it follows that the program should be limited in scope to that necessary to establish whether or not the dosage is being maintained. The accuracy with which it is desired to establish control will largely determine the size of the sample to be obtained. Assume, for example, that the limits within which the mean of any sample of size N should fall are group Mean \pm 1.0 S.E. (68% range of mean). The standard error of the mean depends upon the size of the sample:

$$S.E. = \frac{\sqrt{N}}{N} S.D.$$

Assume, as an example, a group mean plasma level of 16 micrograms/L and standard geometric deviation of 1.45 to represent the universe and that samples of 9 men are taken for examination,

$$S.E. = \sqrt[3]{1.45} = 1.13$$

and 68% of the means of representative samples of 9 each should fall within the limits of \pm 1.13 or 14 to 18 micrograms. If a probability of occurrence of once in 1000 times is used as the limiting criterion, then the sample means, to be considered representative, must fall within $16 \pm (S.E.)^3 = 16 \pm 1.45$ or 11 to 23 micrograms (a range of $\pm (S.E.)^3$ includes 99.9% of the population). If the mean of a given sample falls below the selected lower limits, it may be concluded that all numbers of the group are not receiving atabrine regularly according to the established regimen.

2. Analytical method.

a. Further work on method is desirable. The procedure as used was satisfactory in our hands, since, with few exceptions, the results were consistent. Nonetheless, it must be remembered that the procedure was carried out under the continual supervision of two investigators with extensive analytical and research experience. Unless workers of equivalent training and experience will be available for the ensuing studies, sufficient work should be done with the method to make it an entirely reliable tool in the hands of technicians. With respect to field investigations in the tropics, the fluorometer now in use is very sensitive to atmospheric conditions. This instrument should be improved for use in hot, humid environments and/or a visual instrument developed in order that the vagaries of the photoelectric element may be eliminated.

b. It is evident from considerations of the transients (see Appendix B - II) that the choice of a bleeding 5 hours after dosage is unfortunate since it lies just between the 2-hour and 8-hour transients where the time variation in the first transient will greatly influence the magnitude of the plasma level observed. A better choice would be 3 hours after dosage when the second transient has reached its peak value and the first has largely disappeared.

3. Partition of atabrine in blood. The possibility has been presented that the whole plasma atabrine level in the individual may in some instances be an unreliable measure of the free atabrine concentration in the plasma. The evidence for this is inconclusive. When a study is set up to investigate the correlation between plasma level and malarial protection it is obviously necessary to have available a reliable index of effective atabrine concentration. It is recommended therefore that further investigation be immediately instituted to establish the reliability of whole plasma concentration as well as other measures of free atabrine concentration in the plasma.

4. Toxicity of Atabrine. The short span of this study and the absence of reactions provide no basis for comment with respect to long-time toxicity. In the considerations of a future controlled study of acute toxicity the entirely negative observations here reported are of significance. The freedom from disturbances may be attributed, in part, to the assurance given the men that the drug would not disturb them and the absence of other psychological factors (fear, etc.) likely to cause gastroenteric tract disturbances.

5. Qualifications. It is to be recognized that the foregoing inferences and implications may be strictly applied only to populations having characteristics like those of the experimental group and under states of health and conditions of environment within the ranges described for this study.

APPENDIX E

TABULATION OF RAW DATA

1. Included in this appendix are the crude data* of all groups for the entire experiment in the following order:

- a. A.R.T.C. Co. B Sect. 1 0.4 gm/wk
 - b. A.R.T.C. Co. C Sect. 1 0.4 gm/wk
 - c. A.R.T.C. Co. B Sect. 2 0.6 gm/wk
 - d. A.R.T.C. Co. C Sect. 2 0.6 gm/wk
 - e. Jungle Group A 0.6 gm/wk
 - f. Jungle Group B 0.6 gm/wk
 - g. Group X Dieaway)
 - h. Group Y Dieaway)
- Rearranged from Jungle Groups A and B

* All Means_G and standard geometric deviations were determined graphically.

APPENDIX E

The tabulated raw data is not included in this copy of the report. It may be obtained upon request addressed to Adjutant, Armored Medical Research Laboratory, Fort Knox, Kentucky.

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